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RESEARCH ARTICLE

Comparison of human platelet lysate and 10% fetal bovine serum on mesenchymal stem cell viability, proliferation, migration and paracrine factor expression

Putri Diana Roza [1,2], Maisarah Effajri Putri [3], Amaliyah Rifdah Zulwa [3], Afifah Marwa Marbun [3], Ulfah Ulfah [4], Nicko Pisceski Kusika Saputra [5], Rahmat Azhari Kemal [6], Arfianti Arfianti [6]

[1]Master of Biomedical Science Program, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia
[2]Department of Biochemistry, Faculty of Medicine, Universitas Muhammadiyah Riau, Pekanbaru, Indonesia
[3]Undergraduate Medical Program, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia
[4]Department of Anatomy, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia
[5]Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia
[6]Department of Medical Biology, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia

Correspondence:
Arfianti
Department of Medical Biology, Faculty of Medicine, Universitas Riau, Pekanbaru, Riau – 28133, Indonesia

Email: arfianti@unri.ac.id

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Abstract

Human platelet lysate (HPL) has been proposed as an alternative source to foetal bovine serum (FBS) for culturing mesenchymal stem cells (MSCs), as it eliminates the risk of xenogenicity and may facilitate clinical application. The purpose of this study was to compare the effects of HPL and FBS on MSC viability, proliferation, migration, and expression of paracrine factors. MSCs were cultured in media supplemented with 10% FBS (control), 5% HPL and 10% HPL. Cell viability was determined by the trypan blue dye exclusion and proliferation was assessed using the Cell Counting Kit-8 (CCK-8) assay. MSC migration was evaluated by wound scratch assay after 24 and 48 h of incubation. The expression of the IL-6, VEGF, and TGF-β, which are paracrine factors, was quantified by quantitative real-time polymerase chain reaction (qRT-PCR). The results showed that there was no statistically significant differences in viability, proliferation, and migration were observed between MSCs treated with 5% and 10% HPL and the control group (10% FBS) for 24 and 48. Expression of IL-6, VEGF, and TGF-β was also similar between MSCs cultured in 5% HPL and those cultured in 10% FBS, indicating equivalent functional properties of the cells under both conditions. HPL at 5% supported MSC growth and paracrine factor expression comparably to 10% FBS in vitro. Therefore, 5% HPL may serve as an effective, xeno-free substitute for 10% FBS in MSC culture for clinical applications.

1. INTRODUCTION

Mesenchymal stem cells (MSCs) are among the most promising cell-based products in regenerative medicine because of their multilineage differentiation potential, immunomodulatory properties, and capacity for tissue repair (1). However, clinical translation is challenging because a single therapeutic dose typically requires tens to hundreds of millions

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of cells (commonly 10-100 million), which can only be obtained through extensive in vitro expansion (2,3). The culture medium is therefore as important as the cells themselves: it must support robust expansion without compromising therapeutic potency (4). For many years, foetal bovine serum (FBS) has been the standard supplement, providing complex mixture of growth factors, hormones, and nutrients that supports high proliferation rates. Its animal origin, however, limits its suitability for clinical manufacturing. FBS shows batch-to-batch variability and carries risks of transmitting xenogeneic antigens, bovine prions, and viruses (5). Regulatory bodies have accordingly encouraged a shift toward animal-free alternatives, and the European Medicines Agency recommends avoiding animal-derived materials in the production of Advanced Therapy Medicinal Products (ATMPs) (6,7).

Human platelet lysate (HPL) has attracted particular attention as a xeno-free substitute for FBS. Platelet activation and lysis release a rich mixture of growth factors, including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF) (8-11). These bioactive substances play a major role in proliferation and tissue regeneration (1). Because HPL is of human origin, it also avoids the xenogeneic immunogenicity associated with FBS (12). Several studies report that 5% HPL supports MSC proliferation at a rate comparable to 10% FBS in both bone marrow and adipose tissue-derived MSCs (13). Other studies indicate improved proliferation while stemness and differentiation potential are maintained in HPL-cultured MSCs (14-16).

However, the optimal HPL concentration for MSC culture remains debated. Some researchers recommend 10% HPL (10,17), whereas others report that concentrations of 2-5% yield comparable results for certain outcomes (18,19). This variability underscores the need for comparative studies and standardised protocols for clinical use. In addition, most previous research has focused on proliferation, with less attention given to other migration capacity and paracrine secretion. These properties are critical for therapeutic efficacy, since MSCs must reach sites of injury and release bioactive molecules that promote healing and modulate the immune response (10,17).

Paracrine factors such as interleukin-6 (IL-6), VEGF, and TGF-β, are central to MSC therapeutic activity. IL-6 acts as a pro-inflammatory and tissue-remodelling mediator, whereas VEGF and TGF-β promote angiogenesis, cell proliferation and differentiation, and immune modulation (20-22). Understanding how different culture supplements influence the secretion of these factors is important for optimising MSC efficacy. Cost is a further consideration, particularly in developing countries, where affordable cell therapies are urgently needed. Identifying the minimal effective HPL concentration could reduce manufacturing cost while preserving cell quality.

Therefore, this study compared the effects of 5% HPL and 10% FBS on MSC viability, proliferation, migration, and secretion of paracrine factors (IL-6, VEGF, and TGF-β). It was hypothesised that 5% HPL would be non-inferior, or superior, to 10% FBS in supporting these functional properties, which would support its uses as a cost-effective, xeno-free alternative for clinical-grade MSC production.

2. MATERIALS AND METHODS

2.1. Study Design and Cell Culture

This experimental study was conducted at the Department of Medical Biology, Universitas Riau, Indonesia, from 2024 to 2025. Human umbilical cord-derived mesenchymal stem cells (MSCs) at passages 3-5 were used to ensure cellular consistency. Three experimental groups were established: a control group cultured with 10% foetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Waltham, MA, USA), and two treatment groups cultured with 5% and 10% human platelet lysate (HPL). HPL was prepared from expired platelet concentrates that had been stored frozen for approximately six months after expiration. The platelet concentrates underwent three consecutive freeze-thaw cycles, each consisting of freezing at -80°C for 3 hours followed by thawing at 37°C until completely liquefied. No incubation period was applied between cycles.

2.2. Culture Conditions

The base culture medium consisted of Dulbecco's Modified Eagle Medium (DMEM; Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 1% penicillin-streptomycin and 1% L-glutamine (Sigma-Aldrich, St. Louis, MO, USA). Heparin sodium (2 IU/mL; Sigma-Aldrich) was added to HPL-containing media. Cells were maintained at 37°C in a humidified atmosphere 5% CO_2, and the medium was replaced every 2-3 days.

2.3. Cell Viability and Proliferation Assessment

Cell viability was assessed by trypan blue exclusion test at 24 and 48 hours. MSCs were seeded at 1×10^4 cells per well in 24-well plates (Corning Inc., Corning, NY, USA). Cell suspensions were mixed 1:1 with 0.4% trypan blue solution and counted using a haemocytometer (Marienfeld Superior, Lauda-Königshofen, Germany) under an inverted microscope (Olympus CKX41, Tokyo, Japan). Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Kumamoto, Japan). MSCs were seeded at 2×10^3 cells per well in 96-well plates. CCK-8 reagent (10 µL) was added to 100 µL of culture medium per well and incubated for 2 hours at 37°C. Optical density was measured at 450 nm using a microplate reader (BioTek ELx800, Winooski, VT, USA).

2.4. Cell Migration Assay

MSCs were grown to confluence in 6-well plates (Corning Inc.) and standardised scratches were created using a sterile 200 µL pipette tip (Eppendorf, Hamburg, Germany). Wound closure was photographed at 0, 24, and 48 hours using an inverted microscope (Olympus CKX41, Tokyo, Japan) equipped with a digital camera (Olympus DP21, Tokyo, Japan). Wound area was measured using ImageJ software (version 1.53k, National Institutes of Health, Bethesda, MD, USA). Migration rate was expressed as the percentage of wound closure.

2.5. Quantitative Real-time PCR

After 24 hours of incubation, total RNA was extracted using TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Waltham, USA) and RNA concentration was measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, USA). Complementary DNA (cDNA) was synthesised using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific, Waltham, USA). Quantitative real-time PCR (qRT-PCR) was performed on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, USA) using SYBR Green PCR Master Mix. The primer sequences were as follows: IL-6 forward primer 5'-ACTCACCTCTTCAGAACGAATTG-3', reverse primer 5'-CCATCTTTGGAAGGTTCAGGTTG-3'; VEGF forward primer 5'-AGGGCAGAATCATCACGAAGT-3', reverse primer 5'-AGGGTCTCGATTGGATGGCA-3'; TGF-β forward primer 5'-CAATTCCTGGCGATACCTCAG-3', reverse primer 5'-GCACAACTCCGGTGACATCAA-3'; GAPDH forward primer 5'-GTCAACGGATTTGGTCTGTATT-3', reverse primer 5'-AGTCTTCTGGGTGGCAGTGAT-3'. Thermal cycling conditions were 95°C for 15 seconds and 60°C for 1 minute. Gene expression was normalised to GAPDH and calculated using the 2^(-ΔΔCt) method.

2.6. Statistical Analysis

All experiments were performed in triplicate using three biological replicates (minimum n = 9 per group). Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism version 9.0 (GraphPad Software, Inc., San Diego, CA, USA). Data normality was assessed using the Shapiro-Wilk test. Normally distributed data were analysed by one-way ANOVA followed by Tukey's multiple comparison test; non-normally distributed data were analysed by the Kruskal-Wallis test followed by Dunn's multiple comparison test. A p-value < 0.05 was considered statistically significant.

2.7. Ethical Statement

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethical Review Board for Medicine & Health Research, Faculty of Medicine, Universitas Riau, Indonesia. All procedures involving human-derived samples followed institutional biosafety protocols.

3. RESULTS AND DISCUSSION

3.1. Cell Viability Analysis

The viability of MSCs cultured under the different serum conditions was assessed using the trypan blue exclusion at 24 and 48 hours after seeding. Viability exceeded 90% in all groups (Figure 1). At 24 hours, viability was 94.2 ± 4.8% in the control (10% FBS), 96.1 ± 2.3% in 5% HPL and 95.8 ± 3.1% in 10% HPL. At 48 hours, viability remained above 90% in all groups, with not significant differences between them.

These findings agree with earlier studies showing that HPL provides sufficient nutritional support for MSC survival. The high viability in all groups indicates that both HPL concentrations supply the factors needed to maintain cell survival, which is critical in clinical settings where cell viability directly affects treatment efficacy.

3.2. Cell Proliferation Assessment

Proliferation was assessed by the CCK-8 assay, which measure metabolic activity as a proxy for cell number. As shown in Figure 1B, proliferation did not differ significantly among conditions at either time point. At 24 hours, the proliferation index (expressed as a percentage of the control) was 100.0 ± 3.2% for 10% FBS, 108.7 ± 6.4% for 5% HPL, and 105.2 ± 4.1% for 10% HPL. At 48 hours, the corresponding values were 100.0 ± 8.9%, 102.3 ± 7.6%, and 115.4 ± 8.2% for 10% FBS, 5% HPL, and 10% HPL, respectively. There were no statistically significant differences between groups.

These results are consistent with the meta-analysis by Palombella et al. (10), which reported that MSCs cultured in HPL proliferate at rates equal to or greater than those cultured in FBS. The proliferative effects of HPL is attributed to its high content of bioactive molecules, such as PDGF, TGF-β, and bFGF, which are involved in tissue regeneration and cell proliferation. The comparable proliferation between 5% HPL and 10% FBS suggest that a lower HPL concentration may be sufficient to support proliferation, which could reduce production costs without compromising cell quality.

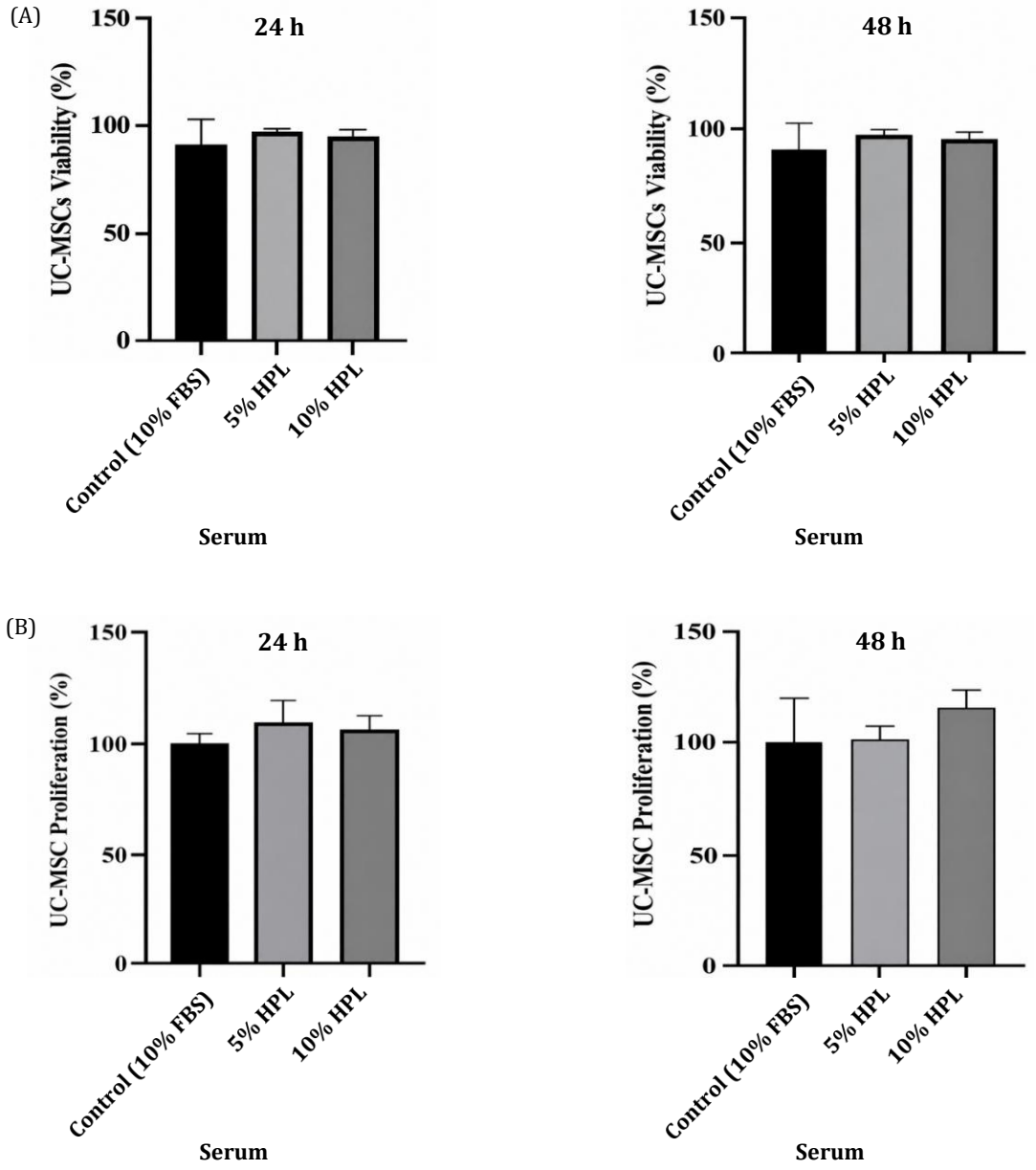

Figure 1. Cell viability and proliferation of MSCs under varying serum conditions. (A) Cell viability determined by Trypan Blue dye exclusion at 24 and 48 hours. (B) Cell proliferation determined by the CCK-8 assay at 24 and 48 hours. MSCs were subjected to control (10% FBS), 5% HPL, and 10% HPL conditions. Values are shown as Mean ± SD (n=9). Statistical analysis: One-way ANOVA, p-value > 0.05

3.3. Migration Capacity Evaluation

Cell migration, a process essential to tissue repair, was evaluated using the wound scratch assay. Representative images of wound closure and the quantification of migration rates are shown in Figure 2. At 24 hours, wound closure was 48.3 ± 12.1% for 10% FBS, 56.7 ± 9.8% for 5% HPL, and 53.2 ± 15.4% for 10% HPL. At 48 hours, wound closure was nearly complete in all groups (98.9 ± 2.1%, 95.4 ± 8.7%, and 89.6 ± 12.3%, respectively). Differences between groups were not statistically significant.

Preserved migration capacity in HPL-cultured MSCs is particularly relevant for clinical application, because the therapeutic efficacy of MSCs depends on their ability to migrate to sites of injury and contribute to tissue regeneration. The comparable migration rates suggest that HPL does not impair the cellular machinery required for migration, although this was not examined directly. This finding is relevant to applications such as wound healing, cardiovascular regenerative medicine, and orthopaedics.

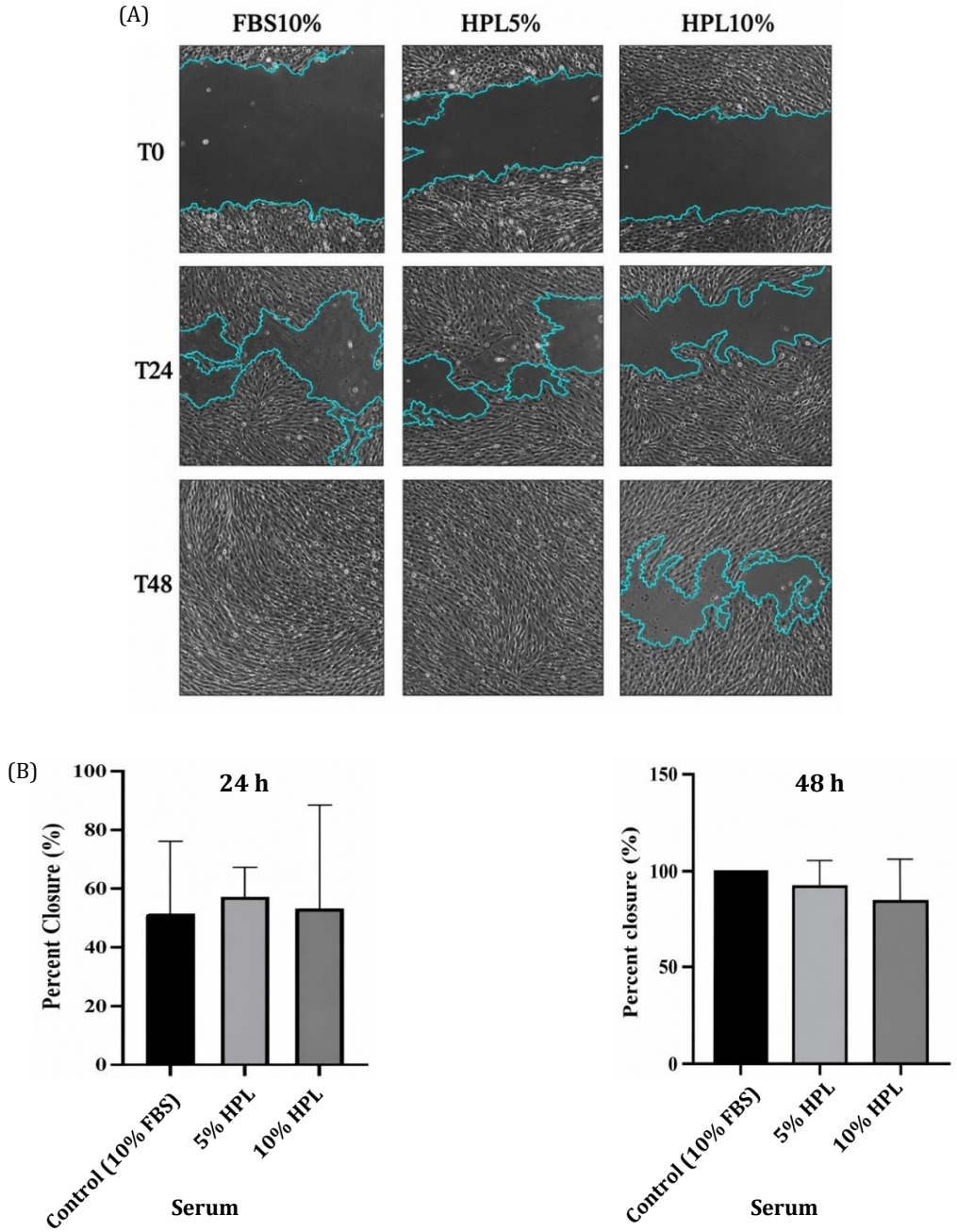

Figure 2. Evaluation of MSC migration potential. (A) Microscopic images representative of wound scratch assays depicting wound healing at time 0, 24, and 48 hours under various serum environments; (B) Statistical results of wound healing percentage at 24 and 48 hours. MSCs were exposed to control (10% FBS), 5% HPL and 10% HPL. Results are expressed as mean ± SD (n=9). Statistical analysis: One-way ANOVA, p-value > 0.05

3.4. Paracrine Factor Expression Analysis

The expression of the paracrine cytokines, IL-6, VEGF, and TGF-β was quantified by real-time quantitative PCR. As shown in Figure 3, expression profiles were similar in FBS- and HPL-cultured MSCs. Relative IL-6 expression was 1.132 ± 0.681 for FBS and 1.178 ± 0.595 for HPL (p-value > 0.05). VEGF expression was 0.992 ± 0.152 for FBS and 0.923 ± 0.292 for HPL (p-value > 0.05), and TGF-β expression was 0.956 ± 0.115 and 1.035 ± 0.245 for FBS and HPL groups, respectively (p-value > 0.05). Maintaining paracrine factor expression is crucial for the therapeutic efficacy of MSCs. IL-6 acts regulate both inflammatory responses and tissue regeneration; VEGF drives angiogenesis and vascular remodelling; and TGF-β modulates immune responses and tissue remodelling. The preserved expression of these factors after in HPL-cultured MSCs suggest that their immunoregulatory and regenerative potential is retained, which is essential for clinical translation.

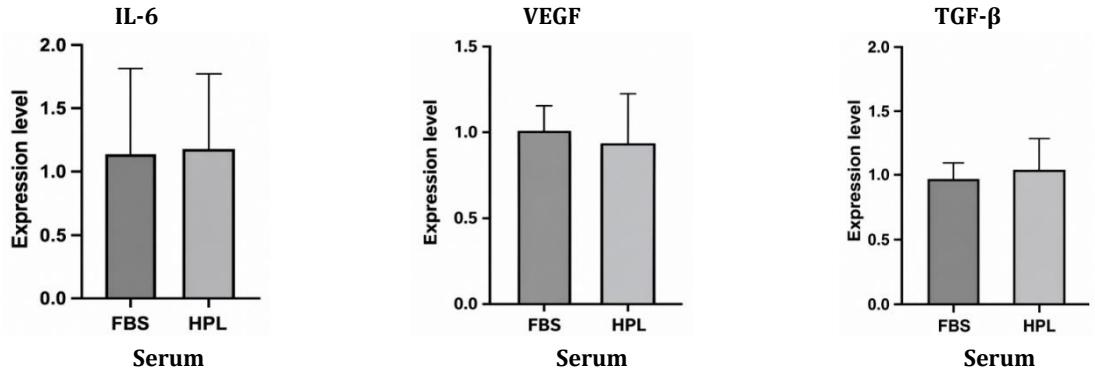

Figure 3. Analysis of paracrine factor expression. Levels of (a) IL-6, (b) VEGF, and (c) TGF-β gene expression detected by qRT-PCR after 24 hours of culture. MSCs were grown in the presence of control media (10% FBS) and 5% HPL. Expression data were normalised to GAPDH expression levels using 2^(-ΔΔCt) method. Values are shown as mean ± SD (n=9). Statistical analysis: One-way ANOVA, p-value > 0.05

3.5. Clinical and Economic Implication

Our results indicate that 5% HPL supports MSC culture comparably to 10% FBS across all parameters tested. This has important implications for the manufacture of cells for clinical use. First, because HPL is free of animal-derived material, it avoids the risk of xenogeneic immune reactions and the transmission of animal pathogens. Second, using HPL at 5% may lower production costs. Third, the use of human-derived supplements is consistent with the European Medicines Agency's recommendations for Advanced Therapy Medicinal Products, which encourage avoiding animal-derived materials in their manufacture. The functional similarity observed here supports a transition from FBS to HPL in protocols for clinical-grade MSC production.

3.6. Study Limitations and Future Directions

Despite being a comprehensive demonstration of the suitability of HPL as an alternative to FBS, there are several limitations that should be considered. First, only a single MSC source was used, and the findings should be confirmed with MSCs from other tissue sources and donors. Second, the effects of long-term culture in HPL and the differentiation capacity of the cells were not evaluated. Third, batch-to-batch variability of HPL, particularly in industrial scale production, remains to be assessed. Further research should therefore focus on optimising HPL preparation to ensure batch consistency, and on evaluating how different HPL concentrations affect the immunomodulatory and differentiation capacities of MSCs. Comparing HPL with other xeno-free alternatives would also help define optimal culture conditions for clinical MSC production.

4. CONCLUSIONS

This study shows that 5% human platelet lysate (HPL) supports for mesenchymal stem cell (MSC) viability, proliferation, migration, and paracrine factor expression comparably to 10% foetal bovine serum (FBS). Therefore, 5% HPL is a promising xeno-free alternative for clinical-grade MSC culture, offering comparable cellular function while eliminating xenogeneic risks and potentially reducing production costs.

Author contributions: PDR, A: Conceptualization. PDR, MEP: Methodology. PDR, AMM, ARZ, MEP, U, NPKS, RAK, A: Investigation. PDR: Formal analysis, Writing – original draft. A: Writing – review & editing, Supervision. All authors read and approved the final manuscript.

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Conflict of interest: The authors declare no competing conflicts of interest.

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