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Assessment of the Dose-Dependent Effect of Human Platelet Lysate on Wharton's Jelly-Derived Mesenchymal Stem/Stromal Cells Culture for Manufacturing Protocols

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Introduction: Mesenchymal stem/stromal cells (MSCs)-based products have unique characteristics compared to other drugs because of their inherently variable effects depending on culture conditions and microenvironment. In some cases, cells can be produced individually, one batch at a time, for personalized therapy. Therefore, it is very important to optimize both culture conditions and medium composition under Good Manufacturing Practice (GMP) standards. MSCs properties have been exploited as potential cell therapies in regenerative medicine. The main mechanism of their protective and regenerative effect is based on their secretory activity. Simultaneously, their secretome is highly variable and sensitive to any change in environmental conditions. Depending on the type of damage and the target application, it is desirable to enhance the secretion of therapeutic factors. Changes in the modulation of environmental conditions can affect survival, migration ability, and both proliferative and clonogenic potentials.

Materials and Methods: This study cultured Wharton's jelly-derived MSCs (WJ-MSCs) in media with varying concentrations of human platelet lysate (hPL). Two groups were created: one with low hPL concentration and another with a high hPL concentration. The effects of these different hPL concentrations were analyzed by assessing mesenchymal phenotype retention, secretory activity, clonogenic potential, proliferation, and migration capabilities. Additionally, the secretion levels of key therapeutic factors, such as Hepatocyte Growth Factor (HGF), Brain-Derived Neurotrophic Factor (BDNF), and Chemokine Ligand 2 (CCL-2), were measured.

Results: WJ-MSCs maintained their mesenchymal phenotype regardless of hPL concentration. However, a higher concentration of hPL promoted cell clonogenic potential, proliferation, migration, and increased secretion of therapeutic factors.

Conclusion: Adjusting the hPL concentration in the culture medium modulates the response of WJ MSCs and enhances their therapeutic potential. Higher hPL concentration promotes increased secretory activity and improves the regenerative capacity of WJ-MSCs, suggesting a promising strategy to optimize MSC-based therapies.

Keywords: mesenchymal stem/stromal cells, human platelet lysate, cell culture, manufacturing protocols, Wharton's jelly

Introduction

Mesenchymal stem/stromal cells (MSCs) can be derived from various sources, including adult tissues such as adipose tissue, bone marrow, dental pulp, as well as postnatal tissues like umbilical cord/Wharton's jelly.[1] Despite the acknowledged capacity of MSCs for self-renewal, proliferation, and differentiation into diverse cell types,[2] the most clinically significant properties are related to the secretory and adjuvant functions.

It has been previously described, that MSCs possess regenerative capabilities associated not only with the repopulation of damaged tissue but also with tissue restoration and modulation of the inflammation process through the secretion of various immunomodulatory factors.[3–5] Many of these secreted molecules, such as cytokines, interleukins, and growth factors play an important role in adjusting in-situ reparative processes. Due to these unique attributes, MSCs seem to be promising candidates for therapeutic applications in diseases resistant to conventional pharmacological treatments.

Received: 23 May 2024
Accepted: 19 September 2024
Published: 5 October 2024

However, for clinical use, the cells must be prepared according to the Good Manufacturing Practice (GMP) standard, following an optimized, established, and reproducible protocol. According to minimal criteria established by ISSCT, MSC must be plastic-adherent when maintained in standard culture conditions. Moreover, MSC must express CD105, CD73, and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR surface molecules, and these cells must differentiate to osteoblasts, adipocytes, and chondroblasts in vitro. Actually, these criteria are expanded according to new research.[2]

The high vulnerability to changes in culture conditions might elucidate the discrepancy between promising results observed in preclinical studies and lack of positive therapeutic effects in clinical trials. The potential cause can be related to the preparation of cell material for clinical trials. There is a wide variation among research groups in the type of cells used, their quantity, the method of administration, and perhaps most importantly, the protocol for their isolation, culture, and large-scale in vitro expansion. Therefore, it is necessary to precisely assess how each of factor influences on the biology and therapeutic attributes of the MSCs and to develop optimal protocols tailored to the specific indication.[6,7]

At the same time, the properties of these change significantly under the influence of various external factors and depend on inter-patient variation. MSCs proliferation, differentiation, and migration potentials are associated with specific environmental conditions like oxygen tension, type of culture or nutrient inflow. An imbalance in the system can contribute to impaired regeneration, uncontrolled cell growth, disruption of tissue homeostasis, or even tumor formation.

The influence of different isolation methods on the MSC properties has been thoroughly investigated. In the case of WJ-MSCs, three primary techniques, enzymatic, explant, and enzymatic-explant,[8–11] have been described. An explant method of isolation appears to be the most optimal as it avoids enzyme contamination, which is important in a clinical trial context. Another clinically significant factor, namely MSCs transportation, has also been optimized and validated. For instance, recent studies have shown that the transport of WJ MSCs in a multi-electrolyte medium ensures higher cell viability than transport in PBS or NaCl.[10]

To amplify the maximum directional potential of MSCs, a variety of cell preconditioning methods are applied. Recent studies have shown that the addition of basic fibroblast growth factor (bFGF) with low initial cell seeding density may increase the expansion of MSCs.[12] Another approach to maintain stemness is the use of three-dimensional (3D) culture conditions. It has been shown that although the paracrine properties of the cells are improved, the spatial conditions impact the early senescence of MSCs.[13,14]

These factors in preclinical studies are provided in FBS. For GMP manufacturing, the FBS and other animal-derived sera are the sources of interspecies contamination such as endotoxins, mycoplasma, viruses, and prion proteins, and as a consequence, zoonotic diseases[15,16] are forbidden. As an equivalent, human platelet lysate (hPL) was introduced.[17] The first reports on hPL are from the 1980s. Umeno et al prepared hPL by repeated freeze/thaw cycles and sonication from fresh blood or outdated platelet concentrates. They showed that hPL supported the proliferation of established cell lines and primary fibroblast.[18] A rich collection of bioactive molecules is found in human platelet lysate (hPL). These encompass growth factors: BDNF, platelet-derived growth factor (PDGF), EGF, and vascular endothelial growth factor (VEGF). Within platelet lysate neurotransmitters like serotonin and histamine, are pivotal for fostering the growth of new neurons. It is also comprised of anti-inflammatory cytokines like transforming growth factor-β (TGF-β), various chemokines, as well as essential antioxidants. This fluid further houses extracellular vesicles with the capacity to deliver these influential factors to cells, along with microRNAs (miRNAs).[19]

Recent studies show that the supplementation of hPL can improve the features of MSCs, but few studies are reporting the optimal dose of hPL in WJ-MSCs culture. The goal of our study is to compare mediums with low and high concentrations of hPL. We expect that the manipulation of the medium's composition, especially the appropriate hPL dose selection, can enhance the proliferation, migration, and secretion potential of WJ-MSCs.

Materials and Methods

Cell Culture

Human umbilical cords (UCs) were obtained from full-term deliveries with informed consent from all mothers, in accordance with approval by the ethics committee, guideline KB/213/2016. The 10–15-cm-long UCs were cut with

a sharp, sterile blade into 2 mm slices. Using a biopsy punch (Miltex, GmbH, Viernheim, Germany), small 2 mm^3 fragments of Wharton's jelly matrix (WJ) were then excised from UCs, and transferred to culture dishes (Figure 1). The obtained cells were cultured in a basic growth medium: DMEM (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), human platelet lysate (3% or 10%, PLTGold® Research Grade Human Platelet Lysate, Mill Creek Life Sciences, Inc., USA), penicillin/streptomycin (1%, Gibco, Thermo Fisher Scientific, Waltham, MA, USA), and heparin (0,1%, Sigma Aldrich, Saint Louis, MO, USA) in humidified incubators under 5% CO_2 at 37°C. The experiments were performed according to the scheme shown in Figure 1. All the cells were analyzed between 3 and 6 passages.

Mesodermal Lineage Differentiation

Adipogenesis

After the cells reached a proper confluence, the standard culture medium was changed to a differentiation medium from the Adipogenesis Differentiation Kit (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). After 14 days of differentiation, the cells were fixed with 4% PFA for 15 min and washed with PBS. After that, 60% isopropanol was added for 5 min. Staining was performed with 99% isopropanol and Oil Red O (Sigma-Aldrich, Saint Louis, MO, USA). The solution was diluted in distilled water (3:2) and stained for 5 min.

Chondrogenesis

Cells were cultured according to previously described standard conditions. After removing the culture medium, the cells were detached by accutase and centrifuged. MSCs were then seeded as a 5µL drop/well in a 24-well plate and incubated for 60 min at 37°C. After that, a differentiation medium from the Chondrogenesis Differentiation Kit (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) was added, and the cells were cultured for 14 days. The cells were then fixed with 4% PFA. The fixed cells were then incubated with a 1% solution of Alcian blue, which detects the presence of cartilage glycosaminoglycans (Sigma-Aldrich, Saint Louis, MO, USA) in 0.1 N HCl, for 30 min at room temperature. Excess dye was then rinsed off with 0.1 N HCl.

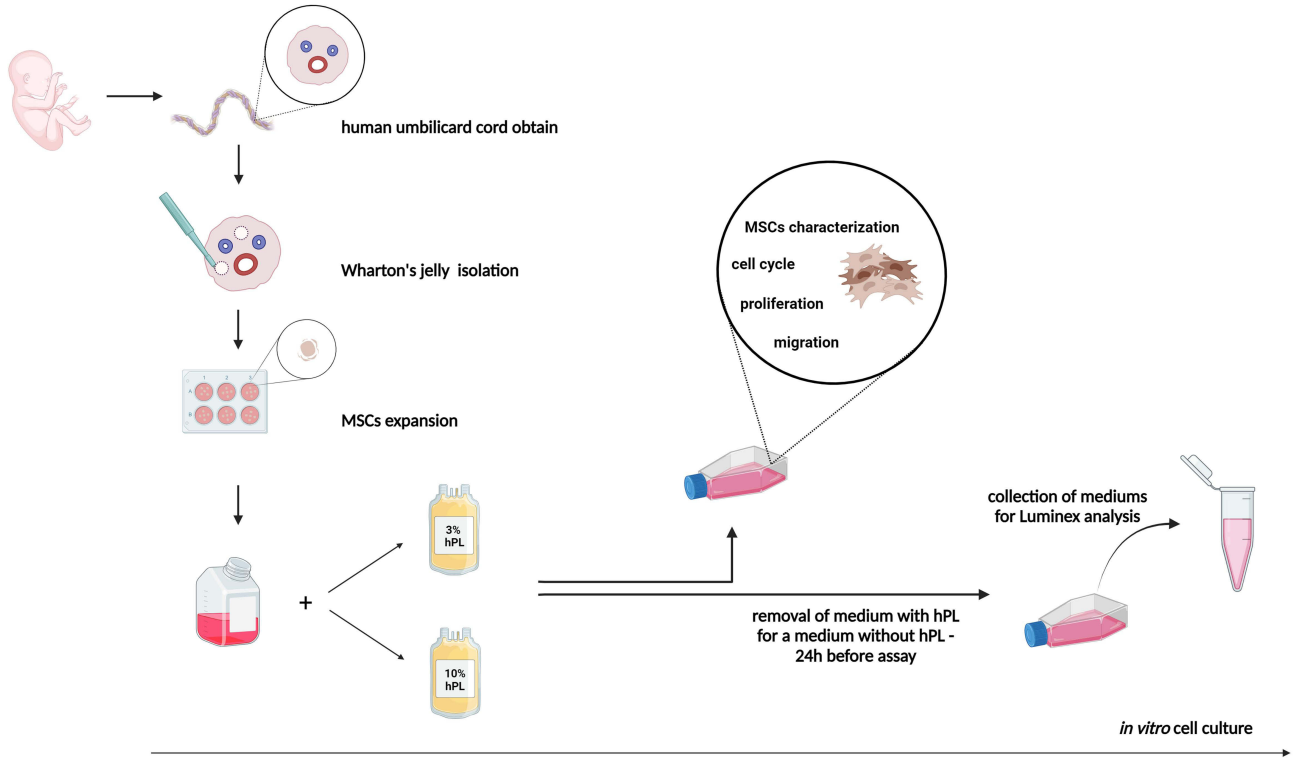

Figure 1 Experiments schema.

Osteogenesis

After the cells reached a proper confluence, the standard culture medium was changed to a differentiation medium from an Osteogenesis Differentiation Kit (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). After 21 days of differentiation, the cells were fixed with 4% PFA for 15 min and then washed with PBS. The cells were washed twice with distilled water and stained with 2% Alizarin Red S (Sigma-Aldrich, Saint Louis, MO, USA). To stain the cells, a dye solution was applied for 3 min before rinsing with distilled water.

CFU-F Assay

The cells at the 4th and 8th passages were seeded on 6-well plates (Nunc, Thermo Scientific) at a density of 10 cells per well and cultured in a medium with 3% (low hPL) or 10% (high hPL) hPL for 10 days. The cells were then fixed with 4% PFA for 15 minutes and stained with 0.5% toluidine blue (Sigma-Aldrich) for 20 min and then rinsed with distilled water. The number of stained colonies with more than 50 cells was counted. CFU frequency was calculated as the number of colonies per number of seeded cells.

Proliferation

Cell proliferation was evaluated using PrestoBlue® assay. The cells were seeded on a 96-well cell culture plate at an initial density of $3*10^3$ cells/cm^2. For the next 5 days, 5 µL of PrestoBlue reagent was added to the wells and incubated for 2 h. Then, absorbance was measured in an immunosorbent assay leader (Spark 10M, Tecan. Mannedorf, Switzerland).

Cell Cycle Analysis

A cell cycle analysis of WJ-MSCs cultured with 3% or 10% of hPL was performed. The cells were fixed overnight in 70% cold ethanol and then incubated with PBS containing 1 mg/mL propidium iodide (Sigma-Aldrich) and 0.1 mg/mL RNase (Sigma-Aldrich) at room temperature in the dark for 30 min. The fluorescence intensities of the DNA-bound propidium iodide in WJ-MSC were measured using a FACS Canto II flow cytometer (BD Biosciences, San Jose, CA), and the results are indicative of the % of total cells in the G1, S, or G2 phase of the cell cycle. Three independent experiments were performed.

Migratory Activity

The influence of 3% or 10% human platelet lysate on WJ-MSCs migratory activity was assessed by scratch assay. WJ-MSCs were cultured in 24-well plates. After reaching 80% confluency, a scratch was made with a 200 µL pipette tip to simulate a wound. The cells were then observed after 2, 4, and 6 hours.

Secretory Activity

Multiplex Human Magnetic Luminex Assay (R&D Systems) was used to measure the protein concentration in medium samples. The concentrations of CCL2/JE/MCP-1, HGF, BDNF, VEGF, or beta-NGF were analyzed using a Luminex-based platform and Luminex 200 IS V2.1 Software (BioRad, Hercules, CA, USA). To eliminate the growth factors and chemokines contained in hPL, we removed hPL from the culture 24 hours before the medium collection. DMEM without hPL and excluding cell cultures was used as a control. Standard curves were generated from the reference factors gradient concentrations. All samples were prepared in this same way; after the medium collection, samples were liquefied and stored at −80°C. All procedures of the media analysis were conducted on the ice. Each sample was frozen/thawed only once.

Statistical Analysis

All the data are expressed as mean ± standard deviation (SD). Comparisons between the two groups were assessed by the Student's *t*-test (CFU-F assay). One-way ANOVA was used to compare among groups. $P < 0.05$ was considered as statistical significance. Statistical analysis was carried out using the statistical software GraphPad Prism 9.1.4 (GraphPad Software, San Diego, CA, USA).

Results

Characterization of MSCs from Wharton's Jelly and Cells Cultured in 3% or 10% hPL

Cells cultured in both hPL concentrations expressed characteristic mesenchymal markers— CD73, CD90, and CD105 (Figure 2A). Isolated WJ-MSCs were able to differentiate into adipocytes—regular red fat drops were present in the cytoplasm of cells in Oil Red O staining. Staining with Alcian blue and Alizarin red confirmed that the WJ-MSCs differentiated into chondrocytes and osteocytes, respectively (Figure 2B). We did not observe any morphological differences between 3% and 10% hPL. Furthermore, we compared the frequency of colony formation unit (CFU-F) in different mediums in the 3rd (early) and 8th (late) passages. We observed significant differences in the clonogenic potential between WJ-MSCs cultured in 3% hPL and 10% hPL. WJ-MSCs supplemented with higher hPL concentration in 3rd passages had 55% ± 12.91 frequency of CFU-F, whereas the cells cultured with lower hPL concentration had 32.5% ± 9.57 frequency of CFU. A similar occurrence was observed in passage 8. WJ-MSC cultured in 10% hPL had 45% ± 5.77 frequency of CFU-F and cultured in 3% hPL concentration had 32.5% ± 5 frequency of CFU-F (Figure 2C).

Differences in a Proliferation and Cell Cycle of WJ-MSCs

The components of the cell culture medium could impact the cell proliferation. Up to the 3rd day of culture, both variant cells reached similar confluence and presented similar proliferation rates and cell cycles. On the 4th day of culture, the

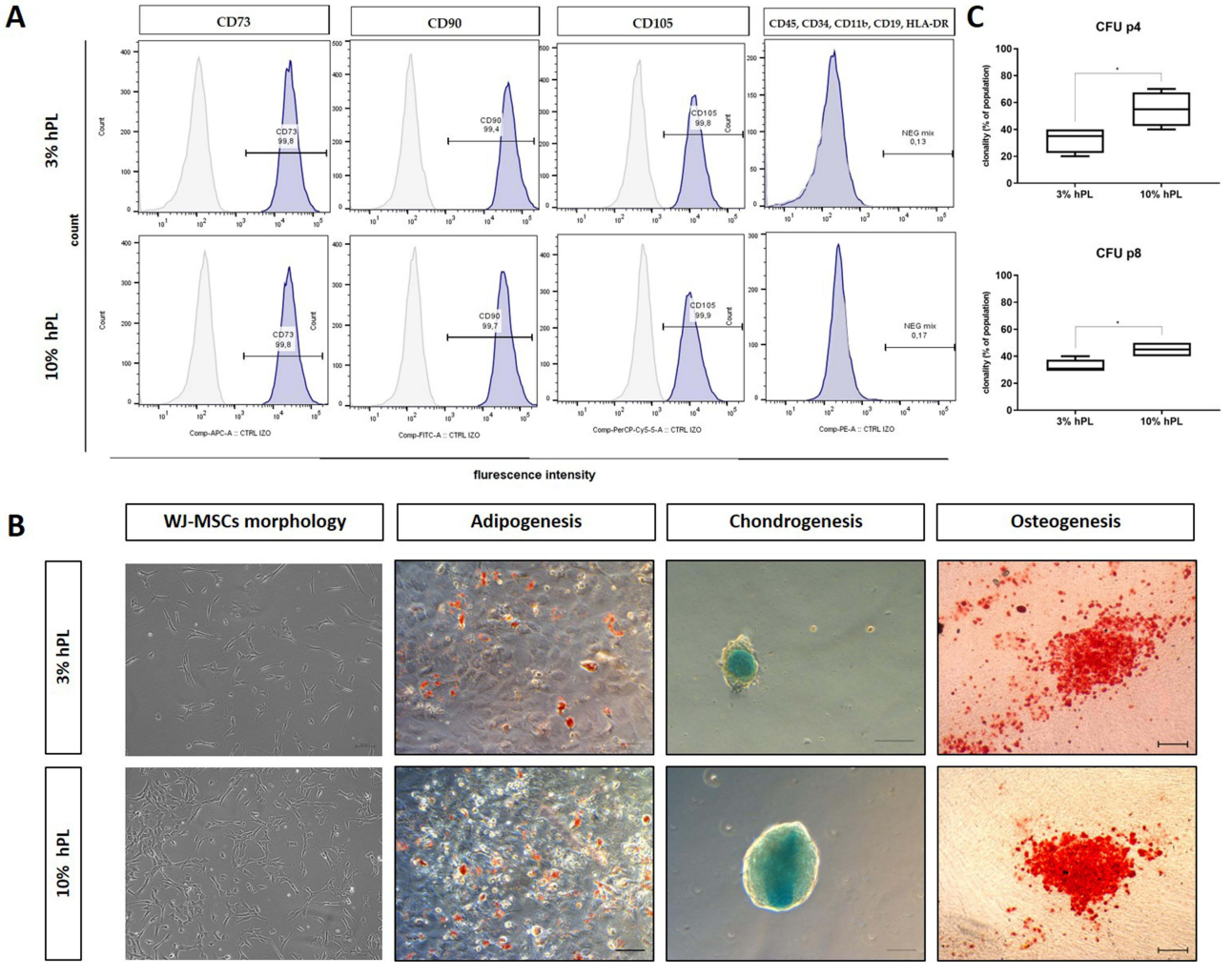

Figure 2 Characterization of cells isolated from Wharton's jelly and cultured in 3% or 10% hPL conditions. (**A**)- MSCs surface markers analysis; (**B**) – CFU-F analysis; (**C**) – morphology of WJ-MSCs, adipogenesis—positive Oil Red O staining (red fat drops), chondrogenesis—positive Alcian blue staining for the presence of cartilage glycosaminoglycans, osteogenesis—positive Alizarin Red staining, scale bar: 50 µm. The results are presented as mean ± SD for each of the donors (n = 3), for * < 0.01.

proliferation rate increased in high hPL concentration conditions. In 10% of hPL cells reached 85% confluency, while cells in 3% hPL reached ~70%. This observation was confirmed by cell cycle analysis. Fourth day of culture, only 64.94% of WJ-MSCs cultured in 3% hPL were in G2/M phase transition, while the cells cultured in 10% hPL were arrested in this phase (4^{th} day: 93.20%; 5^{th} day: 96.11%) due to cell contact inhibition. In 3% hPL conditions, the cell arrest in G2/M phase occurred 24 hours later, on the 5th day of culture. Observations were led for 3rd to 7th day of culture. Despite the differences in the proliferating potential, the proliferating kinetics remained the same (Figure 3).

Rate of Migration of WJ-MSCs in 3% or 10% hPL

For the evaluation of cell motility in different culture conditions, the scratch assay was introduced. In low hPL concentration, WJ-MSCs displayed barely detectable migration after 2 and 4 hours. The presents of 10% hPL enhanced the migration of WJ-MSCs. This effect was observed as early as 2 hours after the beginning of the experiment and exalted for 3% of hPL: 4.1% ±2.8 and for 10% hPL: 14.1%± 3.8. Similarly, after 4 hours of observation, WJ-MSCs cultured in a high concentration of hPL colonized the scratch area faster than cells in 3% of hPL (after 4 h: 3% of hPL 7.5% ±3.3 and 10% hPL 24.4%± 7.5; after 6 h: 3% of hPL 23.1% ±9.4 and 10% hPL 32.8%± 3.3) (Figure 4).

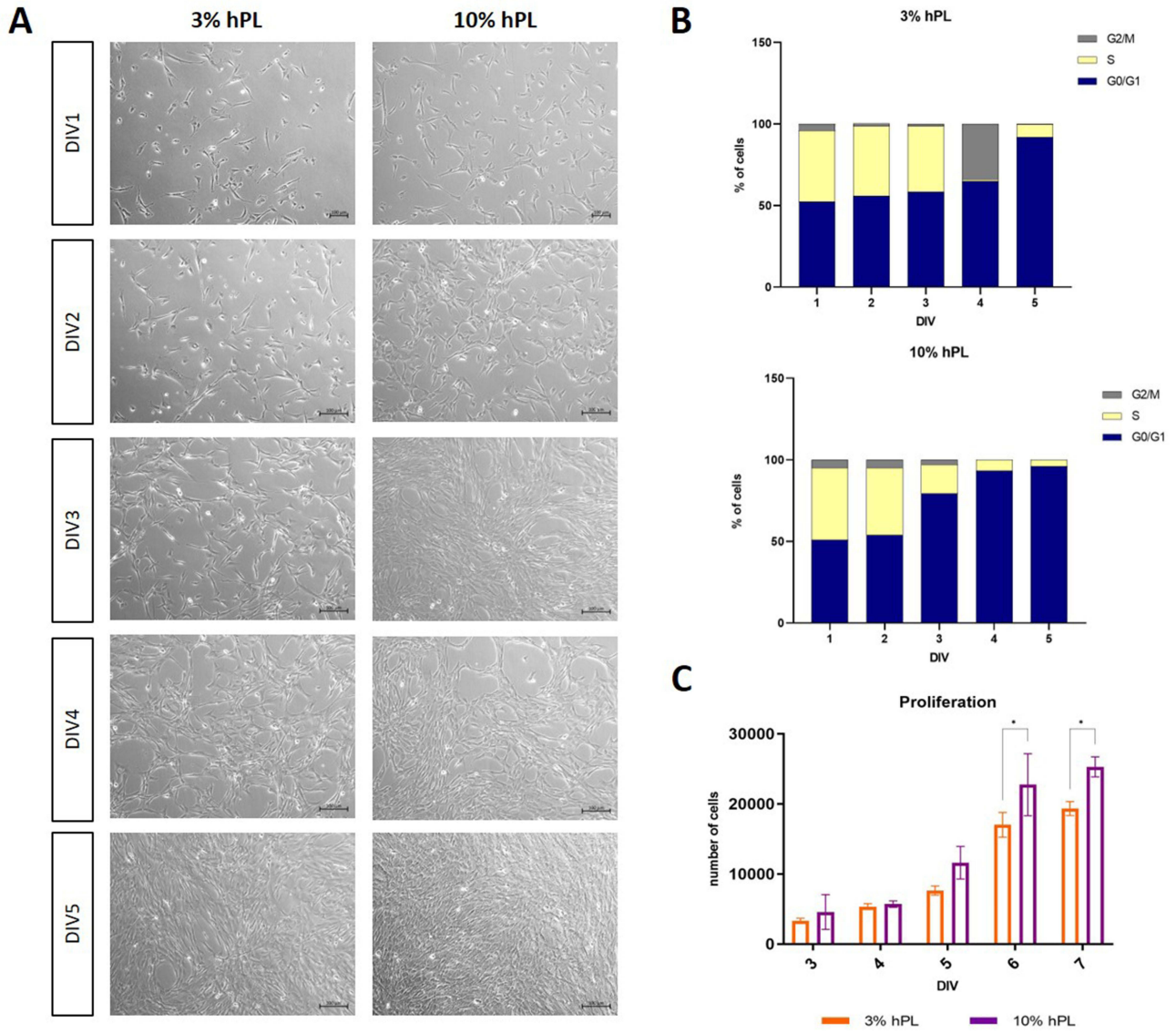

Figure 3 Cell cycle analysis. (**A**)- changes in confluency during 5 days of culture. (**B**) - flow cytometry analysis during 5 days of culture. (**C**) - Comparison of WJ-MSCs 3% vs 10% hPL proliferation. The results are presented as mean ± SD for each of the donors (n = 3), for * < 0.01.

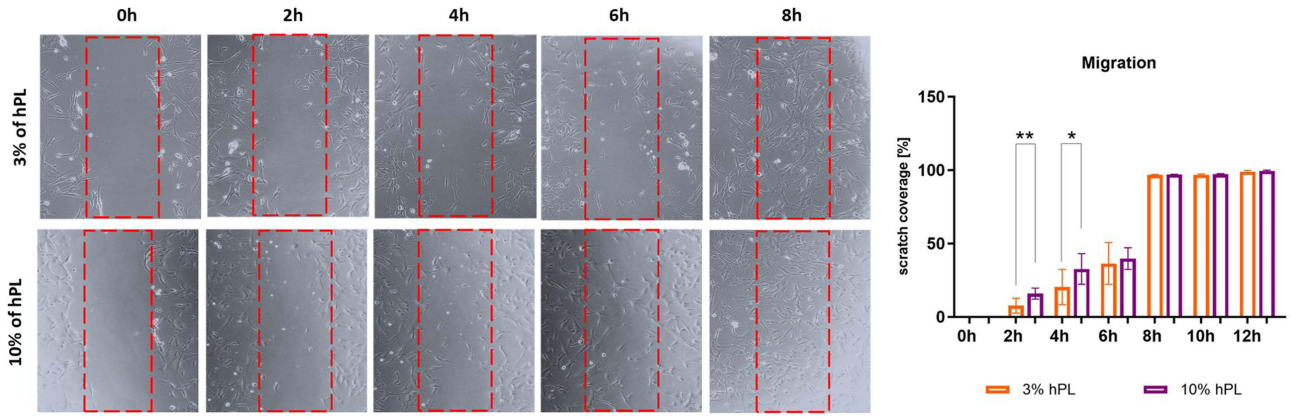

Figure 4 Comparison of migration rate WJ-MSCs cultured in 3% or 10% hPL. The *t*-test between 3% of hPL and 10% of hPL at each time point was analyzed. The results are presented as mean ± SD for each of the donors (n = 3), for * < 0.01, ** < 0.001.

Comparison of the Soluble Secretome of WJ-MSCs Cultured in 3% or 10%

The therapeutic potential of WJ-MSCs is intricately tied to their secretome, encompassing numerous chemokines, cytokines, and growth factors. In this experiment, we analyzed immunomodulatory factor CCL-2, growth factors: HGF and VEGF, and neurotrophic factors BDNF and beta- NGF. The levels of the secreted factors differed between WJ-MSCs cultured in medium with 3% or 10% of hPL. To eliminate the factors contained in hPL from the analysis, it was removed from the culture 24 hours before the medium collection. DMEM without hPL and excluding cell cultures was used as a control.

Significantly higher levels of HGF, BDNF, and CCL2 were observed in cell culture with high hPL concentrations than with 3% hPL. 3% and 10% hPL did not affect the secretion of VEGF and beta-NGF after 24 hours in comparison to the control medium (Figure 5).

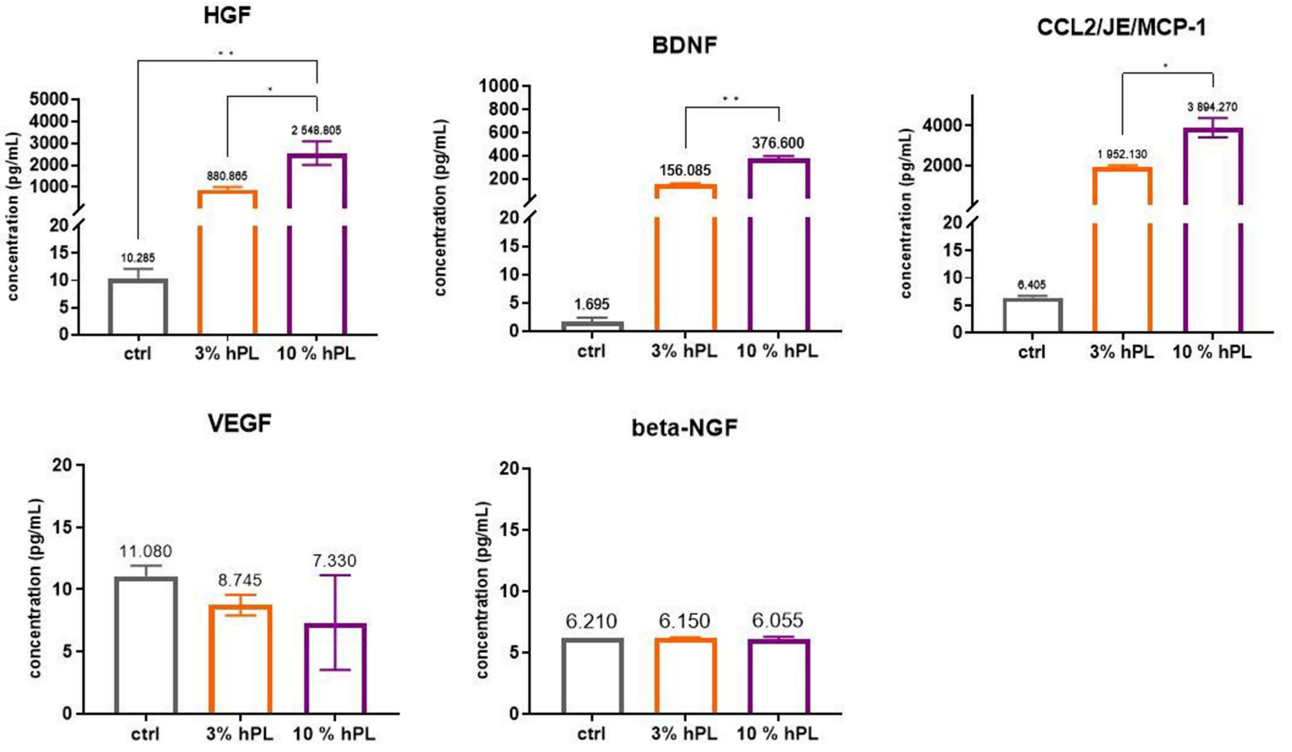

Figure 5 Comparison of the HGF, BDNF, CCL2/JE/MCP-1, VEGF, and beta-NGF secretion of WJ-MSCs pre-cultured in 3% or 10% of hPL. The results are presented as mean ± SD for each of the donors (n = 3), for * < 0.1, ** < 0.01. ctrl - DMEM without hPL and excluding cell cultures; 3% hPL – medium pre-conditioned cell culture with 3% of hPL; 10% hPL- medium pre-conditioned cell culture with 10% of hPL.

Discussion

Despite the ongoing debate on the substantive basis for the application of MSCs in cell therapies, MSCs are currently being used in numerous clinical trials. Properties of MSCs vary between individuals/patients, and they are easily affected by any modification in isolation or cultivation conditions.[20] Some environmental factors such as medium composition, O_2 concentration, or spatial condition (2D vs 3D) could drastically affect on cell culture parameters. Moreover, both high sensitivity to any changes in cell culture conditions and strict requirements of cell-based drug production in GMP standards require optimization of the cell culture environment. An optimal, standard (not dedicated for a specific application) protocol for the preparation of cell-based product for clinical administration should not affect the basic mesenchymal features ie phenotype, marker expression, or mesodermal three lineage differentiation.

The hPL is a very important component of cell culture medium, as it contains numerous factors that have a significant impact on cell viability, migration, and their secretory potential. Human platelet lysate has been introduced in place of animal-derived serum such as fetal bovine serum (FBS) for safety reasons (eg risks associated with the transmission of zoonotic diseases).

FBS does not only contain signalling and growth factors but has also been described as an essential factor facilitating adhesion of MSCs to culture dishes. A study of human MSCs showed that culture surfaces pre-treated with FBS promote better attachment and proliferation than untreated culture surfaces or culture without serum supplementation.[21] Moreover, bone marrow MSCs cultured without serum required fibronectin for cellular adhesion. Some authors have emphasized that FBS supplementation is necessary for the initial attachment of MSCs during the isolation phase.[22] In our experiments, WJ-MSCs did not require additional pre-treatment of surfaces for isolation and proliferation; moreover, adhesion capacity did not correlate with hPL content.

Some studies have shown the effect of hPL on MSCs' morphology and mesodermal differentiation potential. Russell et al described that isolation of canine MSCs was not successful when 10% PL supplemented medium was used. Moreover, changes in cell morphology were observed after 21 days, and MSCs had a tendency towards spontaneous adipogenic differentiation with loss of osteoblast and chondroblast differentiation capacity.[23] A change in morphology has also been described in canine MSCs cultured in 10% PL. The cells lost spindle formations and were unable to fully dissociate from the culture flask. Kirsh et al demonstrated that the addition of hPL enhanced AD-MSC osteogenic differentiation in a dose-dependent manner. Similarly, cell spreading and proliferation increased in a dose-dependent manner.[24] In our experiments, hPL in both concentrations did not affect cell morphology as well as mesodermal differentiation abilities.[25] Analyzing the above results, it seems that the effect of hPL on cell adhesion, morphology, and mesodermal differentiation is dependent on the source of the isolated cells, both species and origin (perinatal tissue vs somatic tissue).

As previously described for FBS, we also confirmed the impact of hPL on cell growth kinetics. The described effect on MSCs' proliferative potential was dose-dependent and WJ-MSCs cultured with 10% of hPL displayed a significantly higher proliferation rate than cells maintained in 3% of hPL.[26] Similar results were described by Hagen et al – cells, which have been cultured in 10% PL, had a similar proliferation rate to the ones maintained in 10% FBS, but proliferation rate was different at 5% PL and insufficient at 2.5% PL. These results correlated with lower apoptosis in 10% PL.[25,27] However, it is worth emphasizing that at higher concentrations of PL, over 30%, proliferation was negatively impacted.[28]

The positive, dose-dependent effect may be attributed to a higher content of growth factors derived from hPL in the culture media.[29] The delivered factors as a feedback mechanism activate the cells to the increased secretion of further growth factors. However, it is important to note that the higher proliferation rate can have limitations such as early senescence. Becherucci et al demonstrated in their research that grown in the presence of high platelet lysate show greater signs of senescence.[30] The increased senescence in in vitro culture is closely related to the cell cycle inhibition. In our study, culture at 10% hPL resulted in cell cycle inhibition, but due to the lack of change in cell morphology, typical of aging cultures, and high confluence (80–90%), we associated the cycle changes with contact inhibition effect but this issue probably requires more extensive study. A different approach to cell culture was proposed by Lee et al. They compared serum-free media to culture with FBS. Their study, based on ADSC in vitro culture, showed that the cells cultured in serum-free medium showed a shorter population double time (PDT) than ADSCs cultured in medium with FBS. Simultaneously, culture in serum-free medium reduced the cellular senescence of ADSCs compared to FBS.[31] The

discrepancies in the results may often be attributed to the variability between product series, in both FBS and hPL production and different levels of hormones, growth factors, vitamins, or other signaling molecules.

To ensure the successful therapeutic application of MSCs, it is essential to preserve or even enhance their migratory capabilities towards relevant stimuli, to achieve functional engraftment. We observed that an environment rich in hPL enhances cell potential for migration. This effect may result from the paracrine activity of WJ-MSCs.

hPL provides factors and chemokines that are actively taken up by WJ MSCs to support their efficient expansion while preserving their biological and potential therapeutic properties. Some of these factors have been associated with the production of signals responsible for immune regulation, efficient cell engraftment or migration. hPL, but not horse serum or FBS, induces the secretion of pro-angiogenic and pro-survival factors (osteoprotegerin, VEGF-A, HGF), immunomodulatory molecules (IL-6) or chemokines (GRO-alpha) by WJ-MSCs during the expansion phase. These signals are critical for promoting angiogenesis and the generation of reparative M2 macrophages. In our study, we found that the secretion of some factors depended on the concentration of hPL (HGF, BDNF, CCL2/JE/MCP-1), while for others (VEGF, βNGF), no effect was observed. Such a situation implies changes in cell behavior. 10% hPL increased the migratory potential compared to 3% hPL. This effect may be due to changes in HGF secretion. When MSCs were exposed to HGF in in vitro culture, researchers observed cell arrest in the G0/G1 phase and decrease in proliferation with simultaneous increase in migratory capacity.[32] In our experiments, the increase in migratory potential was not accompanied by a decrease in proliferation induced by higher HGF levels. Although we observed a positive correlation between hPL concentration and proliferative potential, the proliferation kinetics decreased during the last 2 days due to high culture confluence, cell contact inhibition and cell cycle inhibition. HGF is also described as a key factor in maintaining the stemness of MSCs by preserving mitochondrial function through the expression of proteins associated with PI3K/AKT, ERK1/2, and STAT3 signaling pathways.[33] In our experiment, the higher level of secreted HGF observed in 10% hPL positively correlated with the clonogenic potential of WJ MSCs. HGF secretion also plays a role in MSC prevention of apoptosis, suppression of fibroblast proliferation, and promotion of angiogenesis and epithelial cell proliferation. VEGF has a similar effect on angiogenesis, although we did not observe any effect of hPL concentration on the level of this factor.[34,35] A similar effect was observed by Amable et al in their studies.[36] In contrast to the levels of hPL, the secretion of VEGF can be influenced by a variety of factors, such as hypoxia or different types of cells.[37,38]

Another factor that has been analyzed is CCL-2. We observed a significant increase in the amount this factor. This chemokine is classically associated with the recruitment of macrophages and monocytes during angiogenesis, but it has a role in improving migration of cells to the injury site in the acute phase of inflammation.[39] MSCs isolated from the umbilical cord with overexpressing of CCL2 displayed increased angiogenesis and endogenous neurogenesis in rats with middle cerebral arterial occlusion.[40] C-C motif chemokine ligand 2 (CCL2) is a member of the C-C chemokine family. Its effects are mediated through the C-C motif receptor 2 (CCR2). Kuang et al showed that overexpression of CCR2 on MSCs improved the targeted migration of MSCs.[41] This may indicate that by increasing the mobility of the MSCs, the therapeutic effect at the site of injury is further enhanced.

In addition to systemic factors, MSCs secrete growth factors responsible for cellular adaptation in a specific tissue. Such factors include β-NGF or BDNF, two neurotrophic factors, that are involved in cell migration and integration in the site of CNS injury. To increase the secretion of these factors, cells are specifically induced, not only by environmental factors but also by genetic factors. Scheper et al proved that BDNF-overexpressing hMSCs protect neurons significantly better from degeneration than native MSCs.[42] In our study, NGF levels remained independent of hPL concentration; however, BDNF levels correlated positively. It should be noted that the final level was not as significantly increased as after genetic modification. The lack of change in the secretion of tissue-specific factors ie NGF may be related to the absence of ambient resident cells in a target tissue that express additional proteins on their surface stimulate MSCs eg the presence on the surface of the nerve growth factor receptor (NGFR/CD271), which is a receptor for NGF, may be required to activate the secretion of this factor.[43]

Conclusions

The hPL is an important factor in MSCs culture, affecting their features and properties. Based on our findings and literature data, the influence depends on the source of the cells, both the species and their origin.

Human platelet lysate (hPL) does not impact the adhesion, morphology, expression of mesenchymal markers, or the mesodermal differentiation potential of Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs), although impact cell viability and activity. The effect of hPL on cells is dose-dependent, with a concentration of 10% being optimal. At this concentration, cell viability and proliferation are enhanced, and secretion of key growth factors such as HGF and BDNF is significantly increased. These results suggest that a 10% concentration of hPL is ideal for supporting the growth, viability, and functional performance of WJ MSCs, making it a suitable choice for therapeutic applications where enhanced cell function is critical. This concentration balances promoting cell health and maximizing their therapeutic potential, thereby optimizing the conditions for their use in regenerative medicine.

Data Availability Statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study. Access to data files and further information is available upon request by contacting Anna Sarnowska, asarnowska@imdik.pan.pl.

Ethics Approval for Human Biological Material

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Warsaw Medical University (date: 11th October 2016, no. KB/213/2016). Our Institute does not have its own Bioethics Committee and we are assigned to a Committee belonging to the Warsaw Medical University.

Funding

This work was supported by the Mossakowski Medical Research Institute Statutory Fund No 4.

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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