Received: 21 May 2019 | Revised: 6 September 2019 | Accepted: 13 September 2019

DOI: 10.1111/iwj.13233

ORIGINAL ARTICLE

IWJ WILEY

In vitro human cord blood platelet lysate characterisation with potential application in wound healing

Paola Losi[1] | **Maria C. Barsotti**[1] | **Ilenia Foffa**[1] | **Marianna Buscemi**[1] | **Carolina V. De Almeida**[1] | **Marco Fabbri**[2] | **Sabrina Gabbriellini**[2] | **Francesca Nocchi**[2] | **Silvia Ursino**[2] | **Patrizia Urciuoli**[2] | **Alessandro Mazzoni**[3] | **Giorgio Soldani**[1]

[1]Laboratorio di Medicina Rigenerativa, Biomateriali e terapie avanzate, Institute of Clinical Physiology, National Research Council, Massa, Italy

[2]Laboratorio di Immunogenetica, Medicina Trasfusionale e Biologia dei Trapianti, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy

[3]Medicina Trasfusionale e Biologia dei Trapianti, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy

Correspondence
Paola Losi, Laboratorio di Medicina Rigenerativa, Biomateriali e terapie avanzate, Institute of Clinical Physiology, National Research Council, Massa, Italy.
Email: losi@ifc.cnr.it

Funding information
Fondazione Pisa, Grant/Award Number: "Prognostic evaluation of the use of three-dimen

Abstract

Platelets contain abundant growth factors and cytokines that have a positive influence on the migration and proliferation of different cell types by modulating its physiopathological processes. As it is known that human umbilical cord blood platelet lysate (UCB-PL) contains a supraphysiological concentration of growth factors, in the present study, we investigated its effectiveness in wound-healing processes. Human UCB-PL was obtained by the freeze/thaw of platelet concentrate (1.1×10^9 platelets/L), and its effect was evaluated on human or mouse endothelial cells, monocytes, fibroblasts, and keratinocytes in different concentrations. Human UCB-PL was observed to have high levels of pro-angiogenic growth factor than peripheral blood platelet-rich plasma. Among the cell lines, different concentrations of human UCB-PL were necessary to influence their viability and proliferation. For L929 cells, 5% of total volume was necessary, while for human umbilical vein endothelial cell, it was 10%. Cell migration on monocytes was increased with respect to the positive control, and scratch closure on keratinocytes was increased with respect to serum-free medium with only 10% of human UCB-PL. We concluded that the human UCB-PL may be useful to produce a large amount of standard platelet concentrates sufficient for several clinical-scale expansions avoiding inter-individual variability, which can also be used as a functional tool for clinical regenerative application for wound healing.

KEYWORDS

human umbilical cord blood, in vitro characterisation, platelet lysate, wound healing

1 | INTRODUCTION

The wound-healing and tissue regeneration processes occur in four steps: clotting, inflammation, cell proliferation and repair of the matrix, and epithelialisation and remodelling of the cicatricial tissue.[1] These steps are dynamic and complex cascade integrations, which depend on coordinated functions of inflammatory cells, endothelial cells, fibroblasts, and keratinocytes. They are led by multiple cytokines and growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF), and vascular endothelial growth factors (VEGF).[2,3] Naturally released by platelets, growth factors promote cell recruitment, tissue regeneration, matrix remodelling,[4] angiogenesis, and blood vessel maturation.[5] Platelets come from megakaryocytes and have a dense cytoplasmic granulation. One part of the

granule stores growth factors, while the other part carries the bioactive factors for the tissue recovery process, such as serotonin, dopamine, histamine, adenosine, and calcium.[6]

In the last few decades, the platelet has been widely studied, and it was demonstrated that it has a great potential to deliver a high concentration of growth factors and chemo/cytokines in a concentrated volume of plasma. As a result, different fields of medicine, aiming to augment the repair of tissues with low healing ability, have been applying platelet-rich plasma (PRP),[7,8] a preparation of autologous plasma enriched with a platelet concentration above that normally contained in whole blood.[9] Its use speeds up the vascularisation of grafts, reduces post-surgery morbidity, improves the regeneration of different tissues, and reduces the formation of a scar as it accelerates the maturity and regeneration of the epithelium of wound.[10] However, its actual clinical application is restricted to platelet gels obtained from autologous peripheral blood. This demonstrates a significant and practical limitation such as the need for repeated blood collections for multiple platelets which might be difficult or clinically inappropriate for some different categories of patients (eg, elderly hypo-mobile patients, neonates, and children).[11]

Therefore, any other options to minimise variability and produce a large amount of standard PRP sufficient for clinical-scale use are necessary. Among the current proposal alternatives, the most promising for autologous PRP from peripheral blood (PB-PL) appears to be the use of human umbilical cord blood PRP. It has already been demonstrated that the umbilical cord blood platelet lysate (UCB-PL) has great potential for research and therapeutic purposes,[12] other than containing increased levels of growth factors, such as epidermal growth factor (EGF), VEGF, fibroblast growth factor, insulin-like growth factor-1, and interleukins and interferons.[13] Besides, UCB-PL also contains serum albumin, transferrin and fibronectin, mineral, ghrelin, adiponectin, vitamins A and E, and several essential fatty acids.[14,15] It has recently been shown that UCB-PL has a similar effectiveness to PB PRP in promoting cell growth and differentiation, more specifically on dermal fibroblast, which makes it an interesting alternative to the tissue-engineering applications.[16,17]

Based on these statements, we observed the effect of different concentrations of human UCB-PL on endothelial cells, monocytes, fibroblasts, and keratinocytes culture. Therefore, we obtained the UCB-PL following the procedure established by Rebulla et al[12] and demonstrated its effect on the viability, proliferation, and migration of two important cell lines applied in wound-healing research: human umbilical vein endothelial cells (HUVECs) and mouse fibroblasts (L929). Our results showed that UCB-PL might be useful to produce a large amount of standard platelet lysate sufficient for several clinical-scale expansions as it increased cell viability and proliferation when compared with a basal medium in a concentration-dependent response. However, it was also verified that, in high concentrations, UCB-PL could have an inhibitor effect on cell viability and proliferation.

Key Messages

- the wound healing and tissue regeneration processes usually result in scar tissue, which change the skin's essential functions and can moreover result in devastating psychological consequences, reducing the quality of the individual's life
- the aim of our study was to verify if human cord blood (CB) platelets lysate (PL) can improve cell viability and migration on wound-healing assay, and to do that, we tested different concentrations of the lysate in human and murine cell lines culture and compared its viability, proliferation, and migration rates
- we demonstrated that, because of the high levels of pro-angiogenic growth factor present in human umbilical CB-PL, it could improve the viability and proliferation of these cells
- we believe that UCB platelets can become a functional tool for clinical regenerative application for wound-healing process

2 | MATERIALS AND METHODS

2.1 | Ethics statement

The Italian Cord Blood Network has a programme to collect whole blood and prepare donor-derived platelet concentrate and is a structure that has been accredited by the Regional Health Authority. Approval from an institutional review board or ethics committee was thus not necessary. However, written informed consent was obtained from all the parents, and they were informed that the cord blood units not eligible for unrelated transplantation were used for research.

2.2 | Human CB PRP preparation

The CB units were collected in plastic bags (JMS) containing 30 mL of citrate-phosphate-dextrose-adenine-1 anticoagulant according to locally validated standard operation procedures. The units were processed for the preparation of PRP within 48 hours of collection. Only CB units with total nucleated cells $<1.5 \times 10^9$ and platelets $>200 \times 10^6$ were enrolled in this study to obtain a target mean platelet concentration of 1×10^9/L, range 0.8 to 1.2×10^9/L (according with the platelet concentration defined by the Italian Society of Transfusion

Medicine for platelet gel obtained from adult blood), and target mean volume of 10 mL, range 5 to 15 mL.

The PRP was prepared with an initial soft spin centrifugation of CB at 213*g* for 15 minutes, followed by the transfer of PRP into a secondary bag. A second spin centrifugation of the PRP at 2200*g* for 15 minutes was performed to pellet the platelet. Then, we removed the supernatant platelet-poor plasma in excess to adjust the platelet concentration to target value minimum (0.8×10^9/L) and maximum (1.2×10^9/L). Sterility testing performed at the end of the process was negative in all CB platelet concentrate (CBPC). The CBPC units were finally cryopreserved in a −80°C mechanical freezer. A 10 mL plastic bag was used to ensure appropriate storage and transport conditions.

2.3 | Pro-angiogenic growth factor quantification in UCB-PL

The levels of platelet-derived growth factor-AB (PDGF-AB), TGF-β1, and VEGF were assessed in PRP samples using enzyme-linked immunosorbent assays for these factors (Quantikine, R&D Systems, Inc., Minneapolis, Minnesota) according to manufacturer's instructions. Samples for TGF-β1 analysis were acid activated with 1 N HCl. Growth factor concentrations were measured in triplicate in three different samples.

2.4 | Cell culture conditions

PRP biological effect on cell viability, proliferation, migration, and inflammatory response was performed using four different human cell types involved in the different phases of wound healing: monocytes, endothelial cells, fibroblasts, and keratinocytes. THP-1 (human acute monocytic leukaemia cell line, ICLC HTL97014) was maintained at a cell density of 2 to 9×10^5 cells/mL in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 2 mM L-Glutamine, 100 μg/mL streptomycin, and 100 U/mL penicillin. Each experiment was performed on cells that demonstrated viability rates >95% after staining with Trypan blue dye for dead cell exclusion. HUVECs were obtained by treatment with 0.1% collagenase type II (Worthington Biochemical Corporation, Lakewood, New Jersey) and 0.5% albumin solution to allow endothelial cell detachment from the vessel wall.[18] Isolated cells were cultured in Medium 199, supplemented with 20% FBS, 2 mM L-Glutamine,100 μg/mL streptomycin, 100 U/mL penicillin, 100 μg/mL heparin from porcine intestinal mucosa, and 50 μg/mL EGF. Experiments were performed at passage 3 to 4.

The L929 mouse fibroblast cell line (ICLCATL95001) was obtained from connective tissue of C3H. The L929 mouse fibroblast cell line(ICLCATL95001) was obtained from European Collection of Authenticated Cell Cultures (ECACC), and human keratinocytes HaCat (BS CL 168) were cultured in RPMI 1640 supplemented with 10% FBS, 2 mM L-Glutamine, 100 μg/mL streptomycin, and 100 U/mL penicillin. The medium was routinely changed every 3 days, and at confluence, cells were sub-cultured (split ratio 1:3) by trypsinisation (0.5% trypsin/0.02% ethylendiaminetetracetic acid). The culture media described for each cell type were defined as complete media. Experiments on PL effects were performed in the absence of serum and EGF. All the cell cultures were incubated at 37°C in a humidified atmosphere with 5% CO_2 culture media; reagents and serum were purchased from Sigma-Aldrich (Saint Louis, Missouri).

2.5 | The effect of UCB-PL on cell viability

The quick and easy method of tetrazolium-based colorimetric assay with MTT [3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] is used to assess cell viability as a function of redox potential. Actively respiring cells through the succinate dehydrogenase system of the mitochondrial respiratory chain can reduce the water-soluble MTT to an insoluble purple formazan. The formazan is then solubilised, and its concentration is determined by optical density (OD). Briefly, HUVECs (8×10^3 cells/well) were seeded on pre-gelatinised 96-well plates, and L929 (4×10^3cells/well) were seeded on 96-well plates. After 24 hours of incubation, when cell growth was in the logarithmic phase, the medium was carefully decanted and replaced with 200 μL/well of serum-free medium containing 1%, 3%, 5%, 10%, or 20% (v/v) of PL. Complete medium was used as a reference. Cells were allowed to grow for another 72 hours at 37°C; after this, 20 μL of an MTT phosphate-buffered solution (0.5 mg/mL) was added to each well, and cultures were incubated at 37°C for 4 hours. The supernatant was removed from the wells by slow aspiration and replaced with Dimethylsulfoxide (100 μL per plate) to solubilise the MTT tetrazolium dye. At the end of incubation time, the OD was measured at a wavelength of 550 nm using a microplate reader (Spectrafluor Plus; TECAN Austria GmbH, Grödig, Austria). The percentage of cell viability was calculated vs the complete medium (assumed as 100%).

2.6 | The effect of UCB-PL on cell proliferation

BrdU is incorporated instead of thymidine into the DNA of new synthesis, so BrdU incorporation is a suitable method for the quantitative measurement of cell proliferation. Briefly, HUVECs (8×10^3 cells/well) were seeded on pre-

gelatinised 96-well plates, and L929 (4×10^3 cells/well) were seeded on 96-well plates. After that, when cell growth was in the logarithmic phase, the medium was carefully decanted and replaced with 200 μL/well of medium containing PL (1%, 3%, 5%, 10%, or 20%). Complete medium was used as a reference. After 48 hours of PRP incubation, cell proliferation was assessed by BrdU assay (Roche Diagnostics, Mannheim, Germany). The BrdU reagent was added to each well, and after 4 hours of incubation, the assay was performed following the procedure prescription. The OD was measured at a wavelength of 450 nm using the microplate reader. The percentage of cell proliferation was calculated vs the complete medium (assumed as 100%).

2.7 | The effect of UCB-PL on monocyte chemotaxis

Monocyte chemotaxis was measured using a 24-Transwell plate with 5.0 μm pore polycarbonate membrane insert (Corning Costar, Cambridge, Massachusetts). THP-1 cells were resuspended at 1×10^5 cells/mL, and 200 μL of cell suspension was transferred to the upper chamber of each transwell. The lower chamber was filled with 400 μL of medium containing PL at different concentrations (1%, 5%, or 10%). THP-1 cells were allowed to transmigrate for 2 hours in a humidified atmosphere with 5% CO_2. Cells migrated through the polycarbonate filter pores in response to chemoattractant stimuli and were then counted by the Bürker chamber under light microscopy.

2.8 | The effect of UCB-PL on cell migration

PL effect on wound healing-associated migration was assessed by using scratch-wounded keratinocyte monolayer models (scratch closure assay).[19] HaCat were seeded into 24-well plates at a density of 20×10^4, cultured to confluence, and scratched by scraping with a 10 μL pipette tip. Following phosphate buffered saline washes, cultures were re-fed with 500 μL/well of PL diluted at different concentrations (1%, 5%, and 10%) in a serum-free culture medium. Control wells received a serum-free medium or complete culture medium. At 10, 20, and 30 hours after scratching, digital images of cells were captured by a phase-contrast microscope (Axiovert 25, Zeiss, Milan, Italy; O.M. 50X) equipped with a digital camera (EOS 1000D, Canon, Milan, Italy). Scratch closure was qualitatively analysed at each time point with respect to time 0.

2.9 | Statistical analysis

All the experiments were performed at least in triplicate. Data have been presented as mean ± SD. The data were analysed by StatViewTM 5.0 software (SAS Institute, Cary, North Carolina). The means were statistically compared using the independent Student's t test. Values of $p < .05$ were considered statistically significant.

3 | RESULTS

3.1 | UCB-PL has high concentrations of pro-angiogenic growth factor

Mean content of the measured PDGF-AB, TGF-β1, and VEGF doses on UCB-PL were 65 ± 7.1 ng/mL (C.I 58.7–-72.8 ng/mL), 545 ± 66 ng/mL (C.I 479–611 ng/mL), and 3.5 ± 1.4 ng/mL (C.I 2.1–4.9), respectively. Our results highlighted that UCB-PL contains a high amount of growth factors with respect to those from PB-PL (40, 64, and 0.7 ng/mL) according to the literature.[20]

3.2 | UCB-PL influences the cell viability and proliferation

The addition of UCB-PL in low concentrations, such as 1%, 3%, and 5% of total medium volume, was found to increase cell viability and proliferation regarding control conditions (basal medium and complete medium without PL). On the other hand, UCB-PL had inhibited the cell viability and proliferation of L929 and HUVEC cell culture when added in concentrations of 10% and 20%, respectively (Figure 1).

3.3 | UCB-PL increases the monocyte chemotaxis

The UCB-PL effect on cell migration rate was assessed by a chemotaxis assay, using complete culture medium as the positive control (Figure 2); 1% and 5% UCB-PL induced a significant increase ($p < .05$) in migrating cell numbers with respect to serum-free conditions, and 10% UCB-PL produced a significant increase ($p < .05$) when compared with serum-free conditions and a complete culture medium (24 000 and 32 400 cells, respectively, vs 2000, $p < .05$).

3.4 | UCB-PL plays a positive role on HaCat cell migration

The addition of UCB-PL on the medium culture was able to prompt cell migration on the scratch test using HaCat cells. As observed in Figure 3, the best concentration of UCB-PL was 5%. Although the samples that were treated with 1% and 3% of UCB-PL showed no difference to the control, the cells treated with 5% of UCB-PL for 24 and 48 hours showed a higher scratch closure rate.

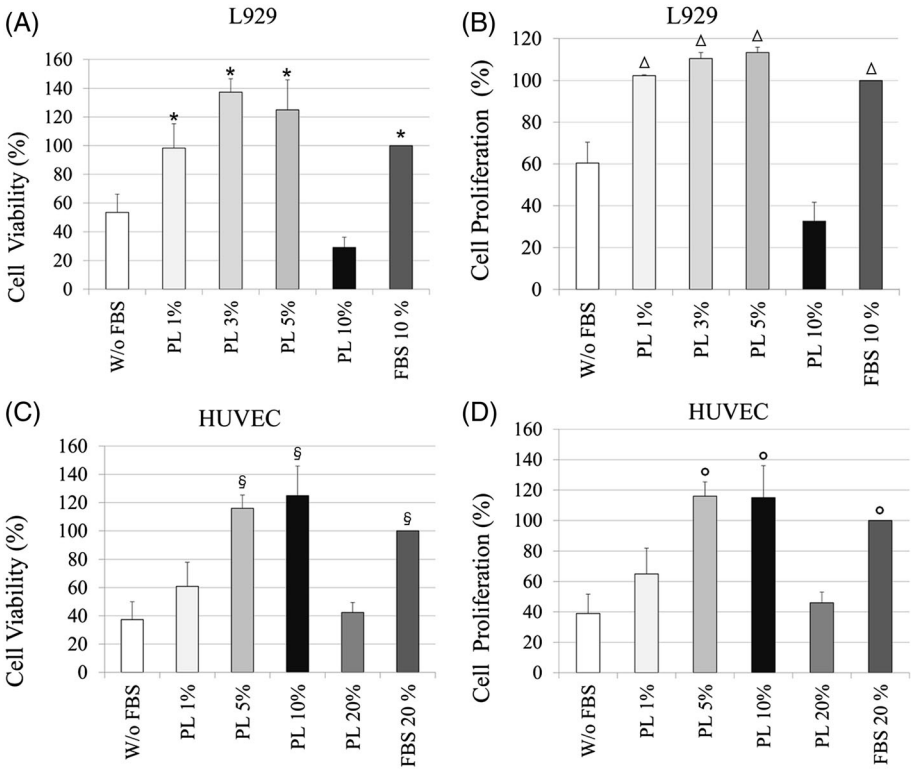

FIGURE 1 Umbilical cord blood platelet lysate (UCB-PL) effect on viability and proliferation of L929 cells and human umbilical vein endothelial cells (HUVECs). Viability (A and C) and proliferation (B and D) were assessed by MTT assay and BrdU incorporation assay, respectively, following 72 and 48 hours of incubation with different UCB-PL concentrations (1%, 3%, 5%, 10%, and 20%). The percentage of cell viability and proliferation were calculated vs the complete medium (assumed as 100%). Data are means ± SD of values obtained from three independent experiments with four replicates each. $^{*}p < .05$ vs both serum-free medium and PL 10%; $^{\Delta}p < .05$ vs both serum-free medium and PL 10%; $^{\S}p < .05$ vs serum-free medium, PL 1% and PL 20%; $^{\circ}p < .05\%$ vs serum-free medium, PL 1%, PL 20%

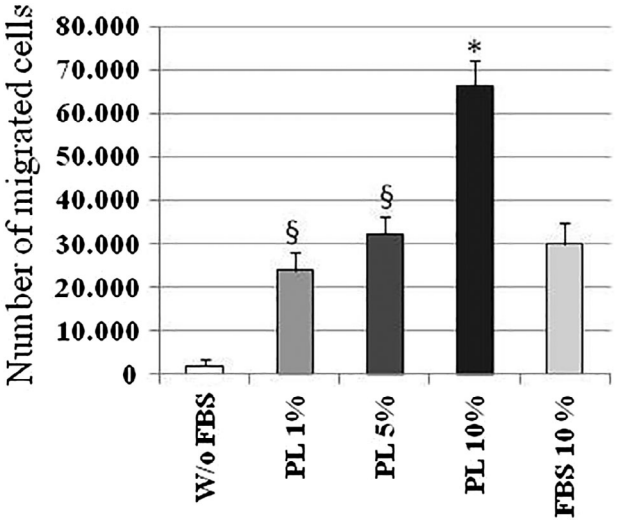

FIGURE 2 The chemotactic effect of umbilical cord blood platelet lysate (PL) on THP-1 cell line was assessed following 2 hours of cell migration towards different PL concentrations (5%, 10%, and 20%). Positive and negative controls are represented by complete culture medium and serum-free medium, respectively. Data (n = 3) are means ± SD of four replicates. $^{\S}p < .05$ vs both serum-free medium; $^{*}p < .05$ vs serum-free medium, 1% PL, 5% PL, and complete culture medium

4 | DISCUSSION

In this paper, we have demonstrated the effect of different concentrations of umbilical cord blood platelets on viability, proliferation, and cell migration in different human and murine cell lines. Our aim was to define the best concentration that, in future, could be applied in protocols to improve the wound-healing process.

In adult mammals, wound healing usually results in scar tissue that can change the skin's essential functions and may have devastating cosmetic and psychological consequences, reducing the quality of an individual's life.[20] In some pathological conditions, such as diabetic skin ulcerations, poor vascular flow can cause the failure of the injured skin reepithelialisation, which will not permit proper wound healing, causing an aggravated scenario with painful lesions.[21] Thus, because of the ability of platelets to stimulate the production of growth factors and cytokines that maximise the healing process at the cellular level, there is an increasing interest in the clinical application of PRP to stimulate the cell recruitment, differentiation, and communication.[22] However, the current clinical trials usually apply platelet concentrates obtained from adult blood to repair topical skin ulcers,[23,24] a protocol that demonstrates significant practical limitations. Given this, the investigation into new

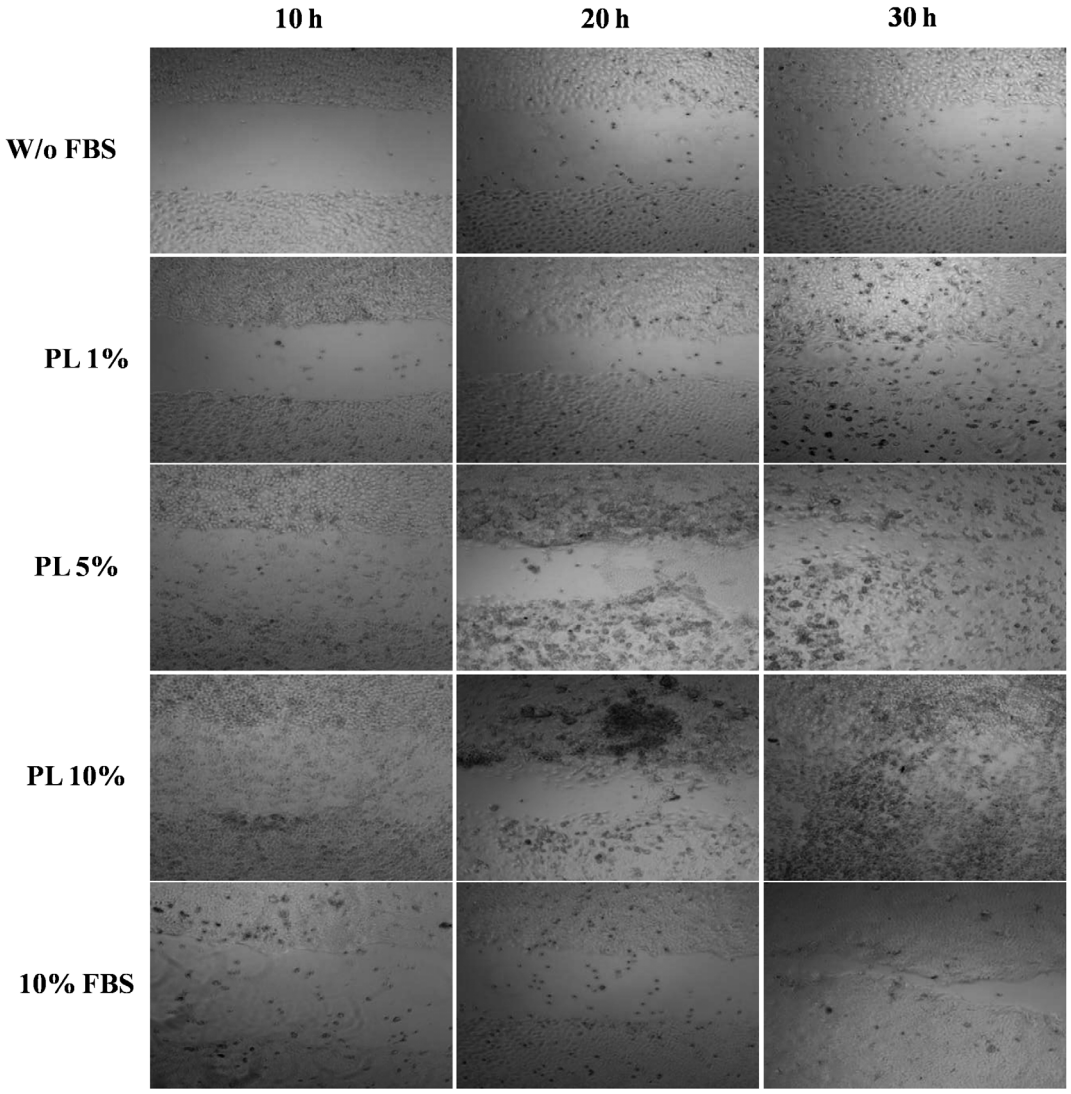

FIGURE 3 Phase-contrast micrographs of scratch test; effect of different concentrations of UCB-PL on HaCat cells migration. The scratch wounds closure was evaluated after 10, 20, and 30 hours of exposure to culture medium added to 10% of FBS and 1%, 5%, and 10% of UCB-PL. All data are representative of three independent experiments with n = 3 by group and are means ± SD

protocols to obtain PRP in a safe and sufficient quantity is required.

Umbilical CB stored in blood banks cannot always be used for haematopoietic stem cell transplantation as up to 80% of stem cells are lower than the cut-off required for such application,[25] being potentially available for a non-transfusion application as a source of growth factor from platelets. Because of the high concentrations of growth factors and cytokines obtained from CB platelet lysates, there appears to be potential for future clinical applications; however, there is still no consensus about the optimal concentrations of UCB-PL that permit clinical application in human trials with no toxic effects. By following the present protocol, we have obtained PL with high concentrations of growth factors, especially TGF-β1 and VEGF. Our results have demonstrated, however, that each cell line responds to different doses of UCB-PL. The murine cell line L929, for example, was responsive with the addition of only 3% of UCB-PL in total medium volume. HaCat, instead, needed 5%, while HUVEC and THP-1 required at least 10% of UCB-PL. Cell lines have also demonstrated to be more or less sensible to induced apoptosis by increasing doses of UCB-PL. For L929 cells 10% of UCB-PL wereenough to cause toxic effects while for HUVEC it was necessarya concentration of 20% to achieve toxicity levels.

PDGF, TGF-β1, and VEGF are fundamental growth factors for a proper wound-healing process as they play essential roles in many processes, such as the proliferation and migration of various cell types, endothelial cell stimulation, angiogenesis, and chemotaxis of fibroblasts and inflammatory cells.[26] The toxic effect that we observed likely correlates with the high concentrations of TGF-β1, which control the

cell growth, cell differentiation, cell proliferation, and apoptosis because of its function as a tumour suppressor.[27,28] It is worth noting that this process of programmed cell death orchestrated by TGF-β1 is fundamental in reducing the excess cell numbers for a successful wound-healing process.[29]

In conclusion, the data suggest that it is possible to use UCB-PL in wound regenerative applications. In particular, we defined the optimal concentration of UCB-PL that could be applied in protocols to improve the wound-healing process. Therefore, our group has been evaluating if the addition of UCB-PL, as a source of growth factors, could improve cell viability and proliferation in skin tissue-engineered substitutes fabricated by 3D bioprinting in order to induce a more rapid tissue maturing.

Although autologous platelets provide safe supplements to treat diseases, the amount of autologous PL is limited and affected by inter-individual variability. UCB-PL may be useful to produce a large amount of standard PL sufficient for several clinical-scale expansions. The data suggest the possibility of using UCB-PL as a tool for clinical regenerative application for wound healing.

ACKNOWLEDGEMENTS

We thank Dr. Thomas Gasson for the English grammar corrections.

This work was supported by Fondazione Pisa through the Project "Prognostic evaluation of the use of three-dimensional (3D) bioactive scaffolds and injectable gels for the treatment of chronic lesions of the skin by means of new biomedical imaging technologies (PREVISION)."

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

P.L. and I.F. participated in the design of the study, and performed the statistical analysis; M.C.B., M.B., and C.V.A. carried out the experimental tests; M.B. and C.V.A. drafted the manuscript; and M.F., S.G., F.N., S.U., P.U., and A.M. carried outperformed all procedures of the UCB-PL production. P.L. and G.S. conceived the study, participated in its design and coordination, and critically revised the manuscript. All authors read and approved the final manuscript.

ORCID

Paola Losi https://orcid.org/0000-0002-1350-8626

REFERENCES

1. Schultz GS, Sibbald RG, Falanga V, et al. Wound bed preparation: a systematic approach to wound management. *Wound Repair Regen*. 2003;11(Suppl. 1:S1-S28.
2. Dulmovits BM, Herman IM. Microvascular remodeling and wound healing: a role for pericytes. *Int J Biochem Cell Biol*. 2012; 44:1800-1812.
3. Anitua E, Sánchez M, Nurden AT, Nurden P, Orive G, Andía I. New insights into and novel applications for platelet-rich fibrin therapies. *Trends Biotechnol*. 2006;24:227-234.
4. Nurden AT. Platelets, inflammation and tissue regeneration. *Thromb Haemost*. 2011;105(Suppl 1):S13-S33.
5. Radziwon-Balicka A, Moncada de la Rosa C, Jurasz P. Platelet associated angiogenesis regulating factors: a pharmacological perspective. *Can J Physiol Pharmacol*. 2012;90:679-688.
6. Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA. Platelet-rich plasma: from basic science to clinical applications. *Am J Sports Med*. 2009;37:2259-2272.
7. Pierce GF, Mustoe TA, Altrock BW, Deuel TF, Thomason A. Role of platelet derived growth factor in wound healing. *J Cell Biochem*. 1991;45:319-326.
8. Pierce GF, Mustoe TA, Lingelbach J, et al. Platelet-derived growth factor and transforming growth factor-beta enhance tissue repair activities by unique mechanisms. *J Cell Biol*. 1989;109:429-440.
9. Malanga GA, Goldin M. PRP: review of the current evidence for musculoskeletal conditions. *Curr Phys Med Rehabil Rep*. 2014;2:1-5.
10. Kevy SV, Jacobson MS. Comparison of methods for point of care preparation of autologous platelet gel. *J Extra Corpor Technol*. 2004;36:28-35.
11. Rebulla P, Pupella S, Santodirocco M, et al. Multicentre standardisation of a clinical grade procedure for the preparation of allogeneic platelet concentrates from umbilical cord blood. *Blood Transfus*. 2016;14(1):73-79.
12. Shamosi A, Mehrabani D, Azami M, et al. Differentiation of human endometrial stem cells into endothelial-like cells on gelatin/chitosan/bioglass nanofibrous scaffolds. *Artif Cells Nanomed Biotechnol*. 2017;45:163-173.
13. Tekkatte C, Gunasingh GP, Cherian KM, Sankaranarayanan K. "Humanized" stem cell culture techniques: the animal serum controversy. *Stem Cell Int*. 2011;2011:504723.
14. Jariwala M, Suvarna S, Kiran Kumar G, Amin A, Udas AC. Study of the concentration of trace elements Fe, Zn, Cu, Se and their correlation in maternal serum, cord serum and colostrums. *Ind J Clin Biochem*. 2014;29:181-188.
15. Dündar NO, Dündar B, Cesur G, Yilmaz N, Sütçu R, Ozgüner F. Ghrelin and adiponectin levels in colostrum, cord blood and maternal serum. *Pediatr Int*. 2010;52:622-625.
16. Hashemi SS, Servatkhah M, Rafati AR. The in vitro effect of different cord blood platelet rich plasma concentrations on proliferation of dermal fibroblasts. *Biosci Biotechnol Res Asia*. 2016;13: 1709-1713.
17. Hashemi SS, Mahmoodi M, Rafati AR, Manafi F, Mehrabani D. The role of human adult peripheral and umbilical cord blood platelet-rich plasma on proliferation and migration of human skin fibroblasts. *World J Plast Surg*. 2017;6(2):198-205.
18. Felice F, Lucchesi D, di Stefano R, et al. Oxidative stress in response to high glucose levels in endothelial cells and in endothelial progenitor cells evidence for differential glutathione peroxidase-1 expression. *Microvasc Res*. 2010;80:332-338.

19. VangMouritzen M, Jenssen H. Optimized scratch assay for *in vitro* testing of cell migration with an automated optical camera. *J Vis Exp*. 2018;(138):e57691.
20. Takeo M, Lee W, Ito M. Wound healing and skin regeneration. *Cold Spring Harb Perspect Med*. 2015;5(1):a023267.
21. Alavi A, Sibbald RG, Mayer D, et al. Diabetic foot ulcers: part I. Pathophysiology and prevention. *J Am Acad Dermatol*. 2014;70 (1):1.e1-1.e18; quiz 19-20.
22. Scherer SS, Tobalem M, Vigato E, et al. Non activated versus thrombin-activated platelets on wound healing and fibroblast-to-myofibroblast differentiation in vivo and in vitro. *Plast Reconstr Surg*. 2012;129(1):46e-54e.
23. Jeong SH, Han SK, Kim WK. Treatment of diabetic foot ulcers using a blood bank platelet concentrate. *Plast Reconstr Surg*. 2010;125:944-952.
24. Picardi A, Lanti A, Cudillo L, et al. Platelet gel for treatment of muco-cutaneous lesions related to graft-versus-host disease after allogeneic hematopoietic stem cell transplant. *Transfusion*. 2010;50:501-506.
25. Screnci M, Murgi E, Carmini D, et al. Related cord blood banking for haematopoietic stem cell transplantation. *Transfus Med*. 2010; 20(3):185-190.
26. Barrientos S, Brem H, Stojadinovic O, Tomic-Canic M. Clinical application of growth factors and cytokines in wound healing. *Wound Repair Regen*. 2014;22:569-578.
27. Gold LI. The role for transforming growth factor-beta (TGF-beta) in human cancer. *Crit Rev Oncog*. 1999;10(4):303-360.
28. Hata A, Shi Y, Massagué J. TGF-beta signaling and cancer: structural and functional consequences of mutations in Smads. *Mol Med Today*. 1998;4(6):257-262.
29. Crowe MJ, Doetschman T, Greenhalgh DG. Delayed wound healing in immunodeficient TGF-beta 1 knockout mice. *J Invest Dermatol*. 2000;115(1):3-11.

How to cite this article: Losi P, Barsotti MC, Foffa I, et al. In vitro human cord blood platelet lysate characterisation with potential application in wound healing. *Int Wound J*. 2020;17:65–72. https://doi.org/10.1111/iwj.13233