International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 15, Issue 4, 2023

Original Article

IN VITRO PROPAGATION OF PLATELETS FROM EMBRYONIC STEM CELLS WITH *C. PAPAYA* LEAF EXTRACT

ADARSH D. B.[1], ELANGO E. MURUGAIAN[1,2*]

[1]Genelon Institute of Life Sciences, Bangalore, India, [2]Evergreen Biosolutions, HIG 162, Avalapalli Hudco, Hosur-635109 Tamilnadu, India
[*]Corresponding author: Elango E. Murugaian; [*]Email: elango.em@gmail.com

Received: 20 Apr 2023, Revised and Accepted: 11 Jun 2023

ABSTRACT

Objective: The depletion of platelets in patients under different clinical conditions like thrombocytopenia is often associated with a significant mortality factor. Alternative modes of platelet propagation from other resources, including stem cells, are evaluated under *in vitro* conditions. Embryonic stem (ES) cells can proliferate and differentiate into various cell types. This study evaluated the generation of functional platelets from embryonic stem cells with *C. papaya* leaf extract through a controlled sequential pathway to meet the clinical demand.

Methods: *In vitro* culture of embryonic stem cells was carried out with the help of growth factors and a suitable medium. The differentiated megakaryocytes and platelets were analyzed by staining, flow cytometer and functional studies. The normal megakaryocytes were compared with the megakaryocytes differentiated from Embryonic Stem cells.

Results: The platelets derived from embryonic stem cells are morphologically identical to normal platelets isolated from peripheral blood, and the number of viable cells was analyzed by flow cytometer. The distinctive two waves of platelet production represented normal primitive and definitive hematopoiesis.

Conclusion: This study is a preliminary milestone in platelet propagation from embryonic stem cells with *C. papaya* leaf extract. The differentiation of ESCs into megakaryocyte-mediated platelets with *C. papaya* leaf extract has shown valuable information on the positive effects that embryonic stem cells can be used for large-scale platelet production.

Keywords: Platelet, Embryonic stem cells, Megakaryocytes, Thrombopoietin, Papaya leaf extract

DOI: https://dx.doi.org/10.22159/ijcpr.2023v15i4.3024. Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr

INTRODUCTION

In normal human blood, the platelet population is 150-400 x 10^3/µl, vital in maintaining homeostasis and atherothrombosis. Due to a short life span (7-10 days), this life-saving blood component is constantly demanded. A shortage of platelets leads to thrombocytopenia in patients under different clinical conditions and is often associated as a significant mortality factor with dengue fever [1-3]. To overcome this difficulty, alternative modes of platelet propagation from different resources, including stem cells, under *in vitro* conditions. Many culture systems demonstrated the maturation of megakaryocytes and the formation of proplatelets from progenitor hematopoietic cells. $CD41^+$stem cells from various sources, including bone marrow, peripheral blood and cord blood cells, have successfully differentiated into megakaryocytes *in vitro* [4-6]. Culture systems, like liquid culture and coculture with stromal cells, require the addition of cytokine, thrombopoietin, IL-3, and IL-6, to release platelets into the culture medium [7, 8]. Such methods have been explored for megakaryocytes or lineage-specific gene expressions. These studies would help therapeutic applications, such as transfusions or cell transplantation.

Embryonic stem (ES) cells are a good source, as these cells can proliferate and can differentiate into various cell types [9]. Embryonic stem (ES) cells are pluripotent cells derived from preimplantation embryos. ES cells can be maintained in culture indefinitely as undifferentiated cell types, yet they are capable of forming more differentiated cell types [10]. Because of these properties, mouse ES cells have been instrumental in gaining a better understanding of mammalian development. However, mouse ES cells differ in morphology, population doubling time and growth factor requirements.

Hematopoietic stem cells (HSC) are adult stem cells with self-renewal and multi-lineage differentiation properties, and thus able to produce diverse cell types such as megakaryocytes. Meanwhile, the bone marrow microenvironment plays an important role in HSC proliferation and differentiation. Also, there is a close contact between the bone marrow niche and megakaryocytes where osteoblast cells, as the main component of the osteoblastic niche, support megakaryopoiesis by releasing different growth factors [11]. Studies carried out over the past two decades have made possible a more exact definition of stem cells, in which they are capable of self-renewal, can differentiate into multiple lineages and will function *in vivo* [12].

Megakaryocytes are mother cells of platelets that are generated *in vitro* from the murine embryonic stem (MES) cells with the development of a coculture system with the stromal cells. In studies of hematopoiesis, investigators have used mouse ES cells to derive various hematopoietic lineages *in vitro* either by the formation of "embryoid bodies," coculture with stromal cell lines, or culture on collagen-coated plates [9, 13]. In contrast to work on mouse hematopoietic development, studies of human hematopoiesis have been confined to primary hematopoietic tissue such as bone marrow, peripheral blood or umbilical cord blood as the starting cell population [14].

It has been evidenced from clinical studies that *C. papaya* leaf extract increased platelet counts in patients suffering from dengue [15-17]. Papaya leaves contain phenolic compounds, papain and alkaloids, and these nutrients act as potent antioxidants, enhancing the body's immunity. The main bioactive compound(s) responsible for the antithrombocytopenic activity of *C. papaya* leaves are alkaloids, particularly the "carpaine" alkaloid, which helps prevent diseases like malaria and dengue. We have earlier reported on platelet propagation from hematopoietic stem cells with *C. papaya* leaf extract [18]. This study evaluated the action of *C. papaya* leaf extract on embryonic stem cell differentiation into platelet.

MATERIALS AND METHODS

Sample

The embryonic stem cells were kindly provided by Stellixir Biotech Pvt Ltd, Bengaluru, Karnataka, India.

Chemicals

The chemicals Dulbecco modified essential medium, fetal bovine serum, thrombopoietin, and penicillin were procured from GIBCO, Thermo fisher, USA. The RNAase was obtained from Sigma. Prostaglandin E1 (PGE1) was from Ono Pharmaceutical, Osaka, Japan. Other chemicals like formaldehyde, acetone, potassium ferricyanide, propidium iodide, acetylthiocholine iodide, sodium citrate, and copper sulfate were purchased from Qualigens, India.

Preparation of papaya leaf extract

The young *C. papaya* leaves were collected from the Somwarpet town area in Karnataka, India cleaned with water to remove contamination. After air drying, they were cut into pieces, pulverized into powder and stored in a polyethylene bag. The powder (45g) was extracted with 350 ml of solvent (ethanol) in a soxhlet apparatus. The final concentrate was evaporated, and 2.5g of final powder was obtained. This was dissolved in 2.5 ml water (1 mg/ml) and used for analysis.

Isolation of human embryonic stem cells

Plated embryonic stem cells were monitored during the first expansion period (Passage0, P0) for several days before the first passage (P1). Cell plating density was set at $6x10^3$ cells per square centimeter in the first passage (P1). Direct cell counts analyzed cell growth to determine the lag, log and plateau phases and population doubling time (PDT). Upon reaching 100% confluence, cells in every 75 cm^2 flask were detached using 0.05% trypsin/0.02% EDTA in phosphate–buffered saline (PBS). Flow cytometric analysis for specific markers, such as CD105, CD44 and CD73, conjugated with either fluoresce in isothiocyanate (FITC) or phycoerythrin (PE), was used. Hematopoietic cells were excluded by sorting for CD45, CD34, and CD14 conjugated with PE.

Culture and differentiation of embryonic stem cells

Embryonic Stem Cells were cultured in Dulbecco modified essential medium (DMEM) supplied with 15% knockout serum and 1% Penicillin and Thrombopoietin (TPO)/*C. papaya* leaf extract (1 mg/ml) at pH7.2. Cell culture was performed from day 0 to day 15 in a 25 cm^3 tissue culture flask at 37 °C in a 5% CO_2 fully humified atmosphere with ES Cells 1165 X 10^3 cells/ml from day 3 of the culture with daily media change till day 15.

Characterization of megakaryocytes differentiated from ES cells

Megakaryocytes derived from ES cells were determined by morphology, immunocytochemistry, Wright-Giemsa and acetylcholinesterase (AChE) staining.

Comparison of CD 41+megakaryocytes from HESCs with that of normal human erythrocytes from peripheral blood samples

Megakaryocyte diameters were measured by comparing $CD41^+$megakaryocytes in the immune-stained preparations and normal human erythrocytes from peripheral blood samples. Microscope photographs were taken at the same magnification (x200), and the mean erythrocyte diameter was calculated as 7 µm.

DNA content of the differentiated cells was analyzed by flow cytometry. Cells were collected from the culture plate by mild pipetting, and the detached cells were labeled with MWReg30 followed by fluorescein isothiocyanate (FITC)–conjugated goat antirat immunoglobulin G (IgG). The cells were washed, resuspended in hypotonic propidium iodide (50 µg/ml in 0.1% sodium citrate) containing 20 µg/ml RNAase, and incubated for 30 min in the dark. The ploidy of the $CD41^+$cells was analyzed with a flow cytometer.

The megakaryocytes originating from both ESC and Human Blood were compared. The extension of culture was carried out till day 13. Size, structural analysis and staining with ACTH and Giemsa were also carried out.

Differentiation of primitive and definitive megakaryopoiesis from embryonic stem (ES) cells

For immunostaining, a cytospin preparation or cultured cells in a 6-well plate were fixed with 4% formaldehyde-acetone solution and stained with anti-mouse CD41 (GPIIb/IIb) monoclonal antibody MWReg30, followed by a polymeric alkaline phosphatase–conjugated secondary antibody. Visualization was performed using a substrate mixture of naphthol AS–BI phosphate sodium salt (Sigma) and a new fuchsin solution, according to the manufacturer's instructions.

AChE staining was performed as described previously. Unfixed cells were incubated in 0.1 M phosphate-buffered saline (PBS) (pH 6.0) containing 0.05% acetylthiocholine iodide, 0.1 M sodium citrate, 30 mmol copper sulfate, and 5 mmol potassium ferricyanide at room temperature for 3 h.

Flow cytometry and determination of the number of platelets derived from ES cells

Free platelets in the culture supernatant were evaluated by a flow cytometer. The culture medium was gently collected, and the large nucleated cells were removed with centrifugation at 150g for 20 min. The cells in the supernatant were fixed with 1% paraformaldehyde for 1 hour. This was centrifuged at 900g for 10 min, and the cells in the pellet were washed with Hanks balanced salt solution with Ca^{2+}(HBSS) containing 1% FBS. Incubation was carried out with 10g/ml anti-mouse glycoprotein (GP) V monoclonal antibody 1C2 (platelet-specific antibody), followed by FITC–goat antiratIg G; each incubation was performed on ice for 1 h. Finally, the cells were washed and analyzed by a flow cytometer. A single platelet gate was created byES-derived platelets similarly.

The number of platelets produced through ES-derived Megakaryocytes was counted by flow cytometer. Culture supernatants were collected from the well on day 15 of differentiation, and cells in the supernatant were stained as above. The cells were finally suspended in 500 µl HBBS. From the supernatant stock, 50 µl aliquots were collected, and platelets were determined using a flow cytometer.

Functional studies of platelets

For functional studies of the ES-derived platelets, the culture medium was changed with the same medium containing 1 µM prostaglandin E1 (PGE1) 1 d before assaying. The collected culture medium was centrifuged at 150g for 20 min. To the supernatant, 1µM PGE1, 1 U/ml apyrase and a 1:9 volume of ACD solution were added and centrifuged at 900g for 10 min. The cells in the pellet were resuspended and washed twice in 85 mmol Sodium citrate, 111 mmol dextrose and 71 mmol citric acid pH 7.0, containing PGE1 and apyrase and then resuspended in a modified Tyrode-HEPES buffer and determined by flow cytometry.

RESULTS

Embryonic stem cells are the ones most effectively differentiated into platelets. This study evaluated ESC cells for differentiation to megakaryocyte-mediated platelets with thrombopoietin and papaya leaf extract as induction factors. The ES cells were initiated with differentiation on day 1 (fig. 1A), most forming isolated colonies with unclear borders of the cells after day 6 (fig. 1B). On day 15, megakaryocyte colonies were formed with individually identifiable large cells (fig. 1C).

The megakaryocytes originating from ES and Human Blood were subjected to size, structural analysis, AChE and Giemsa staining with extended culture till day 15. The phenomenon of the two waves of platelet production representing primitive and definitive hematopoiesis was evaluated with the time course of megakaryocyte maturation. Consistent with the first wave of platelet production from human blood, small megakaryocytes were observed in some colonies around day 8. However, because these small cells rapidly produced proplatelets and disappeared by day 12, they were not likely to be precursors of the megakaryocytes produced after day 12 (fig. 2C). Large megakaryocytes were observed in other colonies, consistent with the second wave. The two megakaryocytes showed distinctive morphologies (fig. 2E and F).

The platelets producing megakaryocytes differentiated from Embryonic Stem cells on days 9 and 13 were positive by immunostaining with an anti-CD41 antibody. By AChE staining, the

cells on day 9 showed a weaker intensity in immunostaining and AChE staining than those on day 13. Furthermore, the size of the day-9 megakaryocytes was smaller than those of days 13 or 15 (fig. 3) (means of diameters: day 9 = 13.1 µm, day 11 = 13.8 µm, day 13 = 20.2 µm, day 15 = 24.2 µm). On day 9, most cells were uniformly about 12 to 15 µm, whereas cells on day 13 were more than 20 µm, and some large megakaryocytes with diameters of more than 30 µm were observed. These results indicated that the cells from the second wave were morphologically close to a mature adult type of megakaryocyte and that the platelets in the first wave were released from qualitatively different small megakaryocytes. The results suggested primitive and definitive megakaryopoiesis from ES cells.

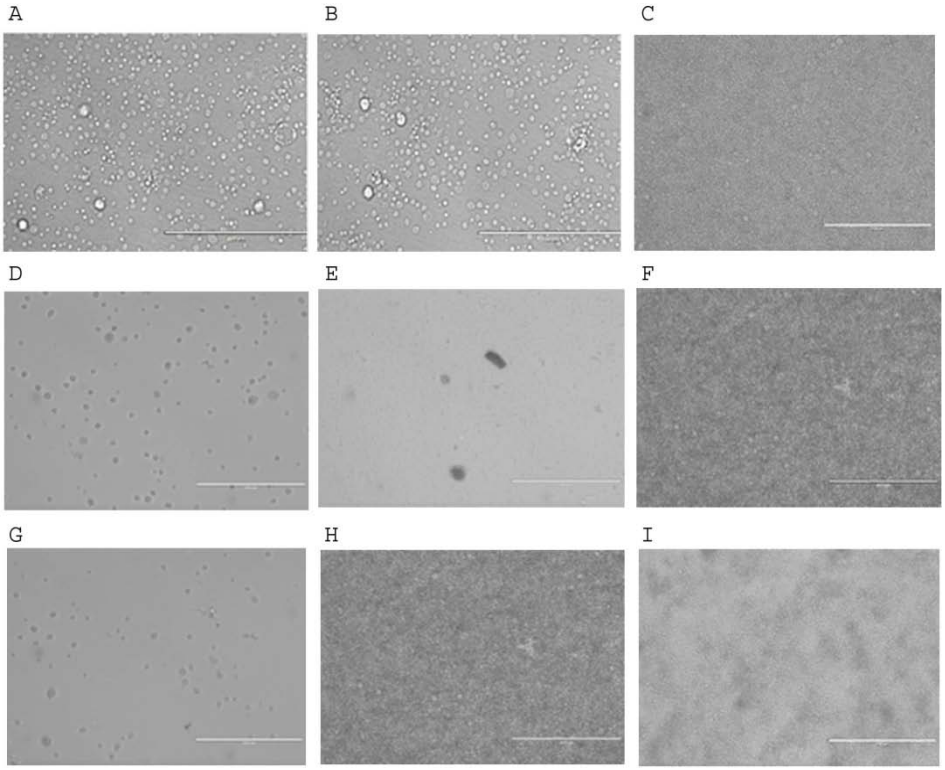

Fig. 1: Culture and differentiation of ES cells with growth factor TPO and *C. papaya* leaf extract. A: Day 1-initiation of cell differentiation: Day 6-ES colony with unclear morphology was formed, indicating initiation of differentiation. C: Day 15-Megakaryocyte colony with individually identifiable large cells. D, E and F: ES cells proliferated without any differentiation in the absence of any growth factors and Day 1, Day 6 and Day 15, respectively. G: Initiation of ES cells differentiation with papaya leaf extract (1 mg/ml). H: Day 6-ESCs proliferated into dense colonies, and the larger colonies progressed into megakaryocytes. I: Day 15-Maturation of megakaryocytes into platelets

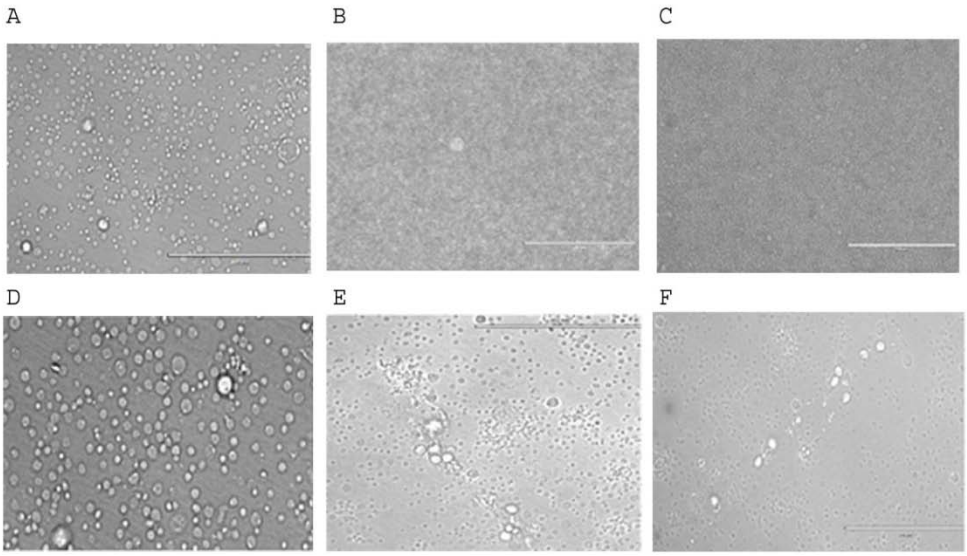

Fig. 2: Characterization of megakaryocytes differentiated from ES cells. A-Day 1 and B-Day 6: ES cells with unclear borders. C-Day 15: ES cells with clear borders. D-Day 1 and E-Day 6: ES cells with unclear borders. F-Day 15: ES cells with clear borders with *C. papaya* leaf extract (1 mg/ml)

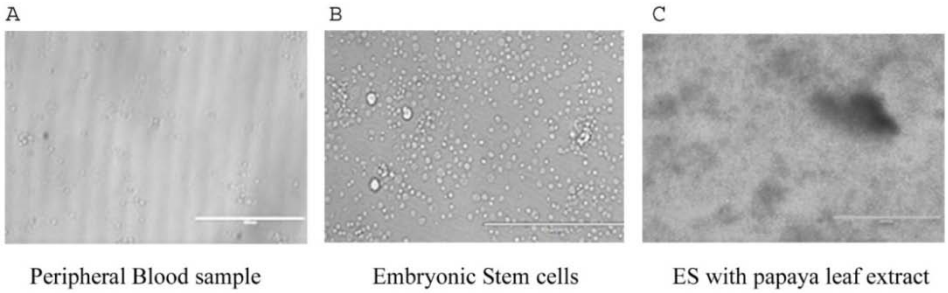

Fig. 3: Comparison of CD 41⁺megakaryocytes from HUCSCs with normal human erythrocytes from Peripheral blood samples. Megakaryocytes were observed under phase-contrast microscopy. Wright-Giemsa staining of the Megakaryocytes differentiated from Peripheral Blood samples. Megakaryocytes differentiated from human blood

To determine that the platelets were produced from the proplatelet-bearing megakaryocytes, the culture medium was collected, and nucleated cells were removed by slow-speed sedimentation. Cells in the supernatant were analyzed by flow cytometer. A gate was fixed in the forward and side scatter profiles of peripheral blood platelets, and most cells in the culture medium of ES cells are within the gate (fig. 4). The size of most cells was within the platelet gate, and almost all cells were positive for CD41 and another platelet-specific antigen, GPV. We conclude that platelets were released to the culture medium from mature megakaryocytes derived from ES cells. The platelets were collected from the medium from day 15, and the number of platelet-sized and CD41⁺cells were accurately counted by flow cytometer (fig. 4C). Large numbers of platelets were produced in the second wave than in the first wave.

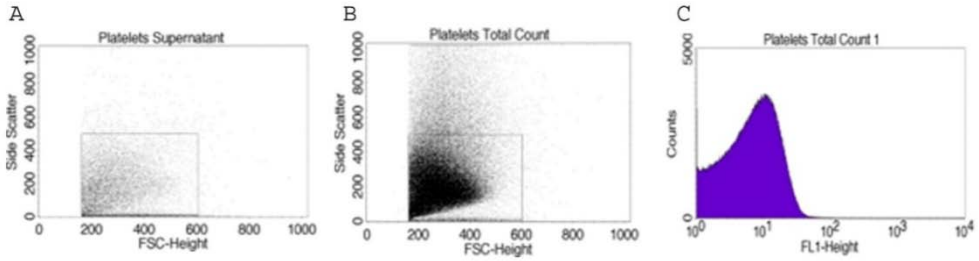

Fig. 4: Flow cytometry analysis for platelets derived from ES cells, cells released (platelets derived from ES cells) to culture medium on day 15 were analyzed by flow cytometry. A platelet gate was fixed in the forward and side scatter profiles of peripheral blood platelets (A) and ES cells (B). Most cells in the culture medium of ES cells are within the gate. Cells in culture supernatant on day 15 were labeled with megakaryocytes and platelet-specific monoclonal antibodies, and almost all cells were positive for CD41 (C)

A functional assay was performed by flow cytometry on the platelets obtained from the second wave of production on day 15. The platelets were shifted to a higher level in the forward scatter after stimulation of PAR4 thrombin receptor activating peptide AYPGFK without stirring, indicating the formation of aggregates (fig. 5). When platelets were stimulated with vigorous stirring, most were shifted to a higher level. Numerous platelet aggregates were observed under microscopy, whereas unstimulated platelets showed no aggregation (fig. 5D and E). Aggregates were probably mediated by fibrinogen binding since the binding of labeled fibrinogen to the platelets was detected. These platelets spread fully and kept a round morphology on the control BSA surface. These results indicated that platelets derived from ES cells *in vitro* were as functional as the platelets from peripheral blood.

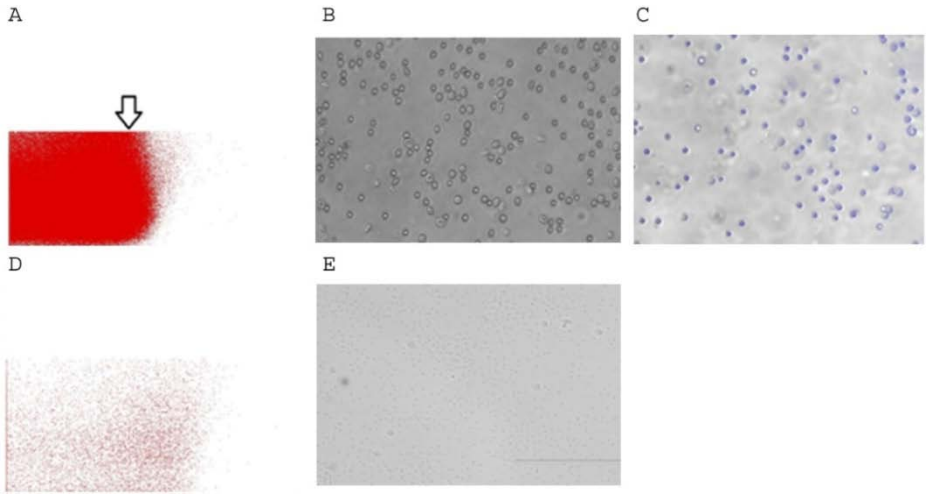

Fig. 5: Functional studies of platelets. In stimulated platelets (+AYPGFK), particles with higher forward scatter are observed (indicating the arrow), indicating aggregate formation (A) and the microscopic image of the analyzed platelet is shown in (B and C). Unstimulated platelets show no aggregation, and a microscopic image of the analyzed platelet is shown (D and E)

DISCUSSION

The human Umbilical cord contains hematopoietic stem or progenitor cells, and they can be maintained for many weeks by culturing. The use of cord blood cells has many advantages: i) it is a disease-free source for hematopoietic cells, ii) the problem of graft-versus-host can be overcome, and iii) the challenge of finding Bone Marrow donors also be overcome. Papaya leaves have been shown to be effective in improving the platelet count in dengue patients and *in vivo* animal models. Since the platelet production is through megakaryopoiesis from stromal stem cells, we evaluated the effect of papaya leaf extract on platelet propagation from cultured embryonic stem cells. In this study, we focused on the isolation of cord blood hematopoietic cells and transforming them intoplatelets producing megakaryocyte progenitors with special reference to *C. papaya* leaf extract. The derivation of HSCs from human ES cells will be more beneficial for human medicine far beyond the treatment of hematologic malignancies, as these HSCs may provide a powerful method to prevent immune rejection of other ES cell-derived tissue [19]. Some researchers have shown a higher harvesting ratio, i.e., the harvesting of MSCs in cord stroma was relatively high (10-15 $X10^3$ cells per centimeter of cord) [19, 20]. This finding is consistent with which MSC yield was reported at 100%, meaning they have harvested cells from every cord received.

HSCs differentiate into mature megakaryocytes through megakaryopoiesis that ultimately produces platelets, critical for hemostasis in the peripheral blood vasculature. The megakaryocytic lineage is derived directly from a common bipotent megakaryocyte-erythrocyte progenitor (MEP). The thrombopoietin receptor (MPL) and its ligand, thrombopoietin (TPO), are essential for the proliferation of megakaryocyte progenitors and their differentiation into mature platelet-producing megakaryocytes [21-23]. Many studies are available on clinical trials and animal models evaluating increased platelets with papaya leaf extract. We have reported platelet production with papaya leaf extract from hematopoietic stem cells through megakaryopoiesis.

CONCLUSION

This study is a preliminary milestone in platelet production by differentiating embryonic stem cells with *C. papaya* leaf extract. The bioactive compounds in *C. papaya* leaf extractplay an essential role in platelet production, protection, and viral pathogen destruction. However, specific compound(s) and their mechanism have not been well established. While most studies were on platelet regeneration and protection under *in vivo* conditions, stem cell-mediated platelet production has yet to be well studied. We initiated the ESCs differentiation into megakaryocyte-mediated platelets with *C. papaya* leaf extract, which has shown valuable information on the positive effects that embryonic stem cells can be used for large-scale platelet production, thus reducing the burden on conventional platelet collection. This has to be further studied to delineate the specific compounds and their action on embryonic stem cell differentiation into platelets.

ACKNOWLEDGEMENT

The authors thankfully acknowledge Averin Biotech Laboratories, Bangalore, India, for the BD FACS CALIBUR for Flow Cytometer.

FUNDING

Nil

AUTHORS CONTRIBUTION

This is the author's sole research work, and each author has contributed equally. This research work does not have contributions from others.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Machlus KR, Italiano JE. The incredible journey: from megakaryocyte development to platelet formation. J Cell Biol. 2013;201(6):785-96. doi: 10.1083/jcb.201304054, PMID 23751492, PMCID PMC3678154.
2. Junt T, Schulze H, Chen Z, Massberg S, Goerge T, Krueger A. Dynamic visualization of thrombopoiesis within bone marrow. Science. 2007;317(5845):1767-70. doi: 10.1126/science.1146304, PMID 17885137.
3. Kowata S, Isogai S, Murai K, Ito S, Tohyama K, Ema M. Platelet demand modulates the type of intravascular protrusion of megakaryocytes in bone marrow. Thromb Haemost. 2014;112(4):743-56. doi: 10.1160/TH14-02-0123, PMID 24965909.
4. Guerriero R, Testa U, Gabbianelli M, Mattia G, Montesoro E, Macioce G. Unilineage megakaryocytic proliferation and differentiation of purified hematopoietic progenitors in serum-free liquid culture. Blood. 1995;86(10):3725-36. doi: 10.1182/blood.V86.10.3725.bloodjournal86103725, PMID 7579339.
5. Hagiwara T, Kodama I, Horie K, Kato T, Miyazaki H. Proliferative properties of human umbilical cord blood megakaryocyte progenitor cells to human thrombopoietin. Exp Hematol. 1998;26(3):228-35. PMID 9502619.
6. Choi ES, Nichol JL, Hokom MM, Hornkohl AC, Hunt P. Platelets generated *in vitro* from proplatelet-displaying human megakaryocytes are functional. Blood. 1995;85(2):402-13, PMID 7529062.
7. Bruno E, Cooper RJ, Briddell RA, Hoffman R. Further examination of the effects of recombinant cytokines on the proliferation of human megakaryocyte progenitor cells. Blood. 1991;77(11):2339-46, PMID 1710149.
8. Bruno E, Cooper RJ, Wilson EL, Gabrilove JL, Hoffman R. Basic fibroblast growth factor promotes the proliferation of human megakaryocyte progenitor cells. Blood. 1993;82(2):430-5, PMID 8329701.
9. Keller GM. *In vitro* differentiation of embryonic stem cells. Curr Opin Cell Biol. 1995;7(6):862-9. doi: 10.1016/0955-0674(95)80071-9, PMID 8608017.
10. Wang Q, Cooney AJ. The role of nuclear receptors in embryonic stem cells. Adv Exp Med Biol. 2013;786:287-306. doi: 10.1007/978-94-007-6621-1_16, PMID 23696363.
11. Fujimoto TT, Kohata S, Suzuki H, Miyazaki H, Fujimura K. Production of functional platelets by differentiated embryonic stem (ES) cells *in vitro*. Blood. 2003;102(12):4044-51. doi: 10.1182/blood-2003-06-1773, PMID 12920021.
12. Morrison SJ, Uchida N, Weissman IL. The biology of hematopoietic stem cells. Annu Rev Cell Dev Biol. 1995;11:35-71. doi: 10.1146/annurev.cb.11.110195.000343. PMID 8689561.
13. Nakano T, Kodama H, Honjo T. Generation of lymphohematopoietic cells from embryonic stem cells in culture. Science. 1994;265(5175):1098-101. doi: 10.1126/science.8066449, PMID 8066449.
14. Kumar PS, Chandrasekhar C, Srikanth L, Sarma PVGK. *In vitro* large scale production of megakaryocytes to functional platelets from human hematopoietic stem cells. Biochem Biophys Res Commun. 2018;505(1):168-75. doi: 10.1016/j.bbrc.2018.09.090. PMID 30243726.
15. Sathyapalan DT, Padmanabhan A, Moni M, P-Prabhu B, Prasanna P, Balachandran S. Efficacy & safety of Carica papaya leaf extract (CPLE) in severe thrombocytopenia (≤30,000/μl) in adult dengue–results of a pilot study. PLOS ONE. 2020;15(2):e0228699. doi: 10.1371/journal.pone.0228699. PMID 32074143.
16. Singh SP, Kumar S, Mathan SV, Tomar MS, Singh RK, Verma PK. Therapeutic application of Carica papaya leaf extract in the management of human diseases. Daru. 2020;28(2):735-44. doi: 10.1007/s40199-020-00348-7, PMID 32367410, PMCID PMC7704890.
17. Sundarmurthy D, RJ, CL. Effect of carica papaya leaf extract on platelet count in chemotherapy-induced thrombocytopenic patients: a preliminary study. Natl J Physiol Pharm Pharmacol. 2017;7(6):1. doi: 10.5455/njppp.2017.7.0202628022017.
18. Adarsh DB, Chandrasagar K, Elango EM. Evaluation of papaya leaf extract in platelet propagation from stem cells. Int J Pharm Pharm Sci. 2023;15(2):54-9.

19. Weiss ML, Medicetty S, Bledsoe AR, Rachakatla RS, Choi M, Merchav S. Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. Stem Cells. 2006;24(3):781-92. doi: 10.1634/stemcells.2005-0330, PMID 16223852.
20. Sarugaser R, Lickorish D, Baksh D, Hosseini MM, Davies JE. Human umbilical cord perivascular (HUCPV) cells: a source of mesenchymal progenitors. Stem Cells. 2005;23(2):220-9. doi: 10.1634/stemcells.2004-0166, PMID 15671145.
21. McRedmond JP, Park SD, Reilly DF, Coppinger JA, Maguire PB, Shields DC. Integration of proteomics and genomics in platelets: a profile of platelet proteins and platelet-specific genes. Mol Cell Proteomics. 2004;3(2):133-44. doi: 10.1074/mcp.M300063-MCP200. PMID 14645502.
22. O'Malley CJ, Rasko JE, Basser RL, McGrath KM, Cebon J, Grigg AP. Administration of pegylated recombinant human megakaryocyte growth and development factor to humans stimulates the production of functional platelets that show no evidence of *in vivo* activation. Blood. 1996;88(9):3288-98. doi: 10.1182/blood.V88.9.3288.bloodjournal8893288, PMID 8896392.
23. Yang J, Luan J, Shen Y, Chen B. Developments in the production of platelets from stem cells (Review). Mol Med Rep. 2021;23(1). doi: 10.3892/mmr.2020.11645, PMID 33179095, PMCID PMC7673345.