BLOOD RESEARCH
VOLUME 58 · NUMBER S1
April 2023
REVIEW ARTICLE

Current status of red blood cell manufacturing in 3D culture and bioreactors

Soonho Kweon[1#], Suyeon Kim[1#], Eun Jung Baek[1,2,3]

[1]*Department R&D, ArtBlood Inc.,* [2]*Department of Translational Medicine, Graduate School of Biomedical Science and Engineering, Hanyang University,* [3]*Department of Laboratory Medicine, College of Medicine, Hanyang University, Seoul, Korea*

p-ISSN 2287-979X / e-ISSN 2288-0011
https://doi.org/10.5045/br.2023.2023008
Blood Res 2023;58:S46-S51.

Received on January 10, 2023
Revised on February 20, 2023
Accepted on March 10, 2023

[#]These authors contributed equally to this work.

*This study was supported by the National Research Foundation of Korea; funded by the Ministry of Science and ICT, Republic of Korea (NRF-2019R1A2C2090053) and by the research fund of Hanyang University (HY-2020).

Correspondence to
Eun Jung Baek, M.D., Ph.D.
Department of Laboratory Medicine,
Hanyang University Guri Hospital, 153
Gyeongchun-ro, Guri 11923, Korea
E-mail: doceunjung@hanyang.ac.kr

Abstract

Owing to donor-related issues, blood shortages and transfusion-related adverse reactions have become global issues of grave concern. *In vitro* manufactured red blood cells (RBCs) are promising substitutes for blood donation. In the United Kingdom, a clinical trial for allogeneic mini transfusion of cultured RBCs derived from primary hematopoietic stem cells has recently begun. However, current production quantities are limited and need improved before clinical use. New methods to enhance manufacturing efficiencies have been explored, including different cell sources, bioreactors, and 3-dimensional (3D) materials; however, further research is required. In this review, we discuss various cell sources for blood cell production, recent advances in bioreactor manufacturing processes, and the clinical applications of cultured blood.

Key Words Erythrocytes, Bioreactors, Erythroid cells, Cell culture techniques

INTRODUCTION

Abnormal production or loss of red blood cells (RBCs) can cause life-threatening anemia and thrombocytopenia. Although blood transfusion is an essential treatment for these patients, inadequate blood supplies and transfusion-related adverse reactions pose potential challenges to the global healthcare system [1, 2]. *In vitro* generation of RBCs has become an important focus to overcome these problems and numerous attempts have been made using different cell types; including human adult hematopoietic stem cells (HSCs), cord blood (CB), bone marrow (BM), peripheral blood (PB), and pluripotent stem cells. Recently, induced pluripotent stem cells (iPSCs) and erythroblast progenitor cell lines have attracted attention owing to their unlimited expansion potential [3, 4].

Since the initial era of 2D culture systems for producing blood cells on a small scale in the laboratory, the use of modified culture systems has been reported. The current consensus is a 3-step method for liquid culture of erythroid cells (Table 1) [5]. Briefly, HSCs are expanded using erythropoietin (EPO), stem cell factor (SCF), and interleukin-3 (IL-3) in a base medium; Iscove's modified Dulbecco's medium (IMDM) or StemSpan serum-free expansion medium (SFEM, Stem Cell Technologies, Vancouver, BC, Canada). Erythroblast expansion in the presence of SCF, EPO, and transferrin is followed by terminal differentiation, with or without EPO. While the expansion of erythroblasts requires supplementation with SCF and EPO, cells at the terminal differentiation stage gradually lose receptors for these cytokines [6]. Since the enucleated reticulocytes still have ferritin

Table 1. Overview of sources and culture media for the production of red blood cells.

	HSC commitment
Cell cultures	Erythropoiesis
Cell sources	CB, BM, PB, hESC, hiPSC, immortalized erythroid progenitor cell lines
Media	StemSpan, IMDM, Cell-Quin[a)]
Additives	EPO, TPO, SCF, IL-3, transferrin, holo-transferrin, IL-6, Flt-3, heparin, IGF-1, glucocorticoids, TGF-β agonist, PPAR-α agonist

[a)]GMP-grade homemade media.
Abbreviations: BM, bone marrow; CB, cord blood; EPO, erythropoietin; Flt-3, feline Mcdonough sarcoma-like tyrosine kinase 3; hESC, human embryonic stem cell; hiPSC, human induced pluripotent stem cell; IGF-1, insulin-like growth factor-1; IMDM, Isocove's modified Dulbecco's medium; PB, peripheral blood; PPAR-α, peroxisome proliferator-activated receptor α; SCF, stem cell factor; IL, interleukin; TGF-β, transforming growth factor β; TPO, thrombopoietin.

receptors; transferrin, which provides iron to erythroid cells, should be supplemented until the end of the final maturation.

Enucleation is a crucial factor because nucleated erythroblasts are less effective at transporting oxygen and are more prone to hemolysis as they pass through small capillaries. Enucleated cells also have the advantage of no DNA and the inability to divide, thus preventing the transfer of malignancy to the recipient [7].

Additional additives, such as thrombopoietin, IL-6, feline McDonough sarcoma-like tyrosine kinase 3, heparin, glucocorticoids, insulin-like growth factor-1, transforming growth factor β agonists, and peroxisome proliferator-activated receptor α agonists, can be added and further modified; with or without serum or feeder layers [8-10].

A major concern is the scale of production required to meet the standard adult therapeutic transfusion dose. Because more than 2×10^{12} RBCs are required per unit, a conventional 2D culture system requires 1,000 L of medium with a culture density of 2×10^{6} cells/mL [11]. High-density cell culture is critical for overcoming the massive volume of media required [5]. Cells grown in suspension should be maintained at a density of $>1\times10^{8}$ cells/mL to produce the numbers required within a minimal medium volume [12]. In addition, greater than 90% enucleation is important for better final erythrocyte yield [10]. Final erythrocytes should have adult hemoglobin and express accurate blood group antigens, such as O RhD-negative, for the universal blood type.

In this review, we discuss recent advances in the manufacturing of RBCs using various cell sources, 3D manufacturing methods, and clinical applications.

CELL SOURCES

The first studies on cultured RBCs (cRBCs) focused primarily on human adult HSCs derived from CB, BM, and mobilized PB [13-15]. However, because of their limited proliferation capacity and low yield, RBCs derived from primary stem cells are difficult to use in clinical settings. An emerging model source uses the high proliferative potential of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). However, these cells show very low degrees of enucleation and very low adult hemoglobin expression. Recent studies have focused on the development of immortalized cell lines at the adult erythroblast stage.

3D MATERIALS AND BIOREACTORS FOR BLOOD CELL MANUFACTURING

Erythropoiesis by mimicking 3D BM niches

Complex 3D culture systems have been studied to mimic the BM niche which regulates the generation of blood cells with a focus on topography, local stiffness of physical constraints, soluble factors, small molecules, and feeder layers.

Severn *et al.* [16] inoculated PB $CD34^{+}$ cells into porous polyurethane scaffolds. These scaffolds exhibited significantly higher total cell egress of hematopoietic progenitor cells (HPCs) than human bone scaffolds (3.90×10^{6} vs. 2.45×10^{6} cells in 1.5 mL of medium). Furthermore, HPCs were released for up to 28 days, achieving a high cell density of 3.7×10^{9} cells/mL. These erythroid progenitors were then cultured with proliferative polymerized high internal phase emulsion (polyHIPE) scaffolds similar to the honeycomb-like structure of the human BM. Cells in this study proliferated 5.18×10^{3}-fold from the starting material and differentiated into 2.59×10^{9} erythroid cells in a spinner flask filled with 1.6 L medium [17].

Lee *et al.* [18] cultured erythroid cells in a 400 μm diameter microporous microcarrier. This increased cell density to 1×10^{7} cells/mL and resulted in a yield of 80% enucleated RBCs. However, when cultured with a microcarrier with a pore size of 30 μm, which is too narrow for erythroid cells to contact each other, the cell viability was too low (8.5%).

Bioreactors for RBC manufacturing

It is unclear which type of bioreactor is the best for RBC production. Culturing at a high cell density is inevitable because of the large quantity of cells required in the final product for transfusion. Perfusion-based hollow-fiber bioreactors are known to increase the cell culture density. In a small scale study using a 2 mL vessel, CB-derived $CD34^{+}$ cells inoculated at a density of 800,000 cells/mL expanded 100-fold and differentiated into erythroid cells over a 7 day period [19]. Additionally, Allenby *et al.* [20] developed a hollow fiber bioreactor and seeded a culture of CB mononuclear cells (MNCs) at a density of 2×10^{7} cells/mL. After 28 days of culture, erythroid cells reached BM-like cell concentrations of more than 2×10^{9} cells/mL. The bioreactor was later modified using a spatiotemporal microenvironmental model that characterized the spatiotemporal distribution of cells, metabolites, and growth factors to guide the design

of an improved version. The modified reactor generated a 50-fold increase in the RBC production efficiency when using a 5-fold lower concentration of growth factors than the previous model [21]. However, despite high-density cell culture using a 3D model mimicking BM niches, the limitation of inefficient oxygen and nutrient distribution need to be overcome for scale-up [22].

Wave-like rocking motion bioreactors, fluidized bed bioreactors [23, 24], rotating wall vessel bioreactors, and stirred tank reactors (STRs) have been proposed for the mass transfer of nutrients and oxygen to achieve greater erythroblast expansion and enucleation.

Since the effects of agitation on *in vitro* erythropoiesis in 24-well plates were studied in 2010 [25], the first 1 L culture in a wave-type bioreactor was demonstrated [26]. When CB-derived $CD34^+$ cells were cultured in a wave-type CultiBag RM bioreactor system (Sartorius, Göttingen, Germany) with 50% dissolved oxygen and a fully defined

Table 2. 3D scaffolds and bioreactors for the production of red blood cells.

Types	Cell source	Culture period	Fold increase in total cells	Enucleation rate	Maximal cell density	Reference
Porous scaffold (polyHIPEs)	PB $CD34^+$ cells	14 days of expansion (in scaffolds), 12 days of differentiation (in spinner flasks)	5,180- fold [(2.59±0.212) $\times10^9$ total cells]	16.25% (post-leuko-filtration)	1.6×10^6 cells/mL (in spinner flasks)	Severn *et al.* [17] 2019
Microcarrier (cytoline I)	Late erythroblasts differentiated CB $CD34^+$ cells	1–3 days after culturing in 2D plates for 13–15 days	2,200-fold in 2D culture	80%	1×10^7 cells/mL	Lee *et al.* [18] 2015
Hollow fiber (BR001)	CB $CD34^+$ cells	7 days	100-fold (small-scale of 2 mL or 8 mL)	40%	8×10^7 cells/mL	Housler *et al.* [19] 2012
Hollow fiber (3DHFR)	CB MNCs	28 days	4.4-fold (550-fold from the stimulated erythroid progenitors)	23%	2×10^9 cells/mL	Allenby *et al.* [20] 2019
Hollow fiber (BR2)	CB MNCs	28 days	50-fold	50%	2.5×10^9 cells/mL	Allenby *et al.* [21] 2022
Wave-type (CultiBag RM bioreactor)	CB $CD34^+$ cells	21 days	1.73×10^6-fold	Not reported	1×10^5 cells/mL	Timmins *et al.* [26] 2011
2 L glass vessels (stirred)	PB $CD34^+$cells	18–24 days	10^4-fold	55–95%	$1–6\times10^5$ cells/mL	Griffiths *et al.* [27] 2012
1.5 L flasks (stirred)	PB and CB $CD34^+$ cells	21 days	10^5-fold	PB: ≤60% CB: ≤38%	$1–4\times10^6$ cells/mL from day14	Kupzig *et al.* [28] 2017
500 mL spinner flasks	hiPSC	39 days	206–805-fold	18.1–59.3%	1.7×10^7 cells/mL	Sivalingam *et al.* [12] 2021
Stirred-tank Applikon BioSep perfusion bioreactor	O-negative hiPSC	29 days	1510.7-fold	≤30%	3.47×10^7 cells/mL	Yu *et al.* [29] 2022
Bottle turning device culture system	CB $CD34^+$cells	21 days	2×10^8-fold	50%	2.42×10^6 cells/mL	Zhang *et al.* [30] 2017
G-Rex bioreactor	PB MNCs	25 days	3×10^7-fold	≥90%	$5–10\times10^6$ cells/mL	Heshusius *et al.* [10] 2019
Stirred micro-bioreactor (Ambr)	CB $CD34^+$ cells	25 days	12-fold	80%	$1–5\times10^6$ cells/mL	Bayley *et al.* [35] 2018
Shake flask	PB MNCs	10 days	13.8-fold	Not reported	3.06×10^6 cells/mL	Lee *et al.* [36] 2018
Stirred bioreactor (Ambr)	CB $CD34^+$ cells	21 days (cultured in 2D plates for 13 days)	2.25×10^4-fold	50%	1.5×10^7 cells/mL	Han *et al.* [37] 2021
Stirred bioreactor (Single wall or AppliFlex)	PB MNCs	22 days	750-fold (0.5 L), 196-fold (3 L)	30–35%	$0.7–2\times10^6$ cells/mL	Gallego-Murillo *et al.* [38] 2022

culture medium, a 2.25×10^8-fold proliferation was obtained; although this was a calculated rather than actual number of cultured cells.

The transfer of static culture to a flask-based system has been studied to scale up culture conditions. Griffiths *et al.* [27] reported that PB $CD34^+$ cells in static culture flasks at a density of $2–10\times10^5$ cells/mL within 500 mL were transferred to a 2 L stirred vessel at a density of $1–6\times10^5$ cells/mL and cultured at 15 rpm. In this system, overall cell expansion was $\geq10^4$ -fold with enucleation rates ranging from 55–95%, corresponding to 5 mL of packed RBCs. The procedure was then scaled up to yield 10 mL of packed RBCs using a good manufacturing practice (GMP)-compliant procedure with $CD34^+$ cells from both CB and PB. Furthermore, they demonstrated the feasibility of large-scale culture in 1.5 L spinner flasks using Bristol Erythroid Line Adult (BEL-A) cell lines [4, 28].

Sivalingam *et al.* [12] demonstrated the scalability of hiPSC microcarrier aggregates on 6-well plates in 500 mL spinner flasks. In a 50-mL culture, the cells were cultured at a high density of 1.7×10^7 cells/mL for erythroid differentiation. Recently, the same group established O-negative hiPSCs in combination with a perfusion bioreactor system capable of high-density cultures of 3.47×10^7 cells/mL of erythroid cells [29].

Zhang *et al.* [30] showed that 1×10^6 CB $CD34^+$ cells cultured in roller bottles produced up to 2.9×10^{11} total cells with a 50% enucleation rate. The amplification of total cells, including unenucleated cells, reached a plateau of approximately 2×10^8-fold by day 21. If all of these cells differentiated into enucleated erythrocytes, the yield of erythrocytes from 1 CB unit (5 million $CD34^+$ cells) could theoretically be equivalent to 500 blood transfusion units; a single unit of transfusable blood contains 2×10^{12} RBCs. Heshusius *et al.* [10] expanded and differentiated $CD34^+$ PB MNC-derived erythroid cells in 1 L gas permeable G-Rex bioreactors with a GMP grade medium. In this system, 3×10^7-fold erythroblast expansion was achieved, yielding 24 mL of packed RBCs and more than 90% enucleation; adult hemoglobin expression and correct blood group antigen expression were identified.

An STR is a cylindrical vessel with a stirrer that is widely used to culture biological agents, including enzymes, antibodies, and cells. It has been widely used in industry because of its relatively easy scale-up, uniform distribution of cells and nutrients, sufficient oxygen transfer, ease of control and monitoring of culture conditions, and compliance with current GMP requirements [31, 32].

The Thomas group [33, 34] used 15 mL Ambr bioreactors using a design of experiments (DOE) methodology. However, the final cell yield was as low as 16-fold after 25 days and 8-fold after 16 days. This group later refined the experimental design for bioreactor process development, gas transfer parameters, and media volume. However, proliferation in the STR was lower than that in static cultures, with 12-fold growth in the STR and 14-fold growth in static cultures being achieved in 25 culture days. Differentiation and enucleation, in addition to a reduction in culture time and media volume, were enhanced in STR compared to static conditions [35]. Lee *et al.* [36] cultured an erythroblast cell line (ImEry) expressing *c-MYC* and *BCL-XL* genes in a 500 mL bioreactor using DOE principles to develop optimized media formulations.

Han *et al.* [37] recently identified the optimal process parameters for differentiating CB-derived erythroid cells in 10–11 mL bioreactors using DOE. Initially, the cells were inoculated into flasks and transferred to the STR. Up to 18 vessels were simultaneously operated to provide reproducible results and the optimal cell seeding density (5×10^6 cells/mL), seeding timing (cell diameter 12–13 μm), pH (7.5), temperature (37°C), agitation speed (500 rpm), medium feeding regimen, and dissolved oxygen were determined. Recently, Gallego-Murillo *et al.* [38] used a single-use STR for expansion and differentiation of PBMC-derived erythroid cells. They scaled up from static cultures to 0.5 L, followed by 3 L cultures with optimal stirring speeds, aeration strategies, and GMP-grade homemade medium (Cellquin). However, further studies are needed to obtain the larger number of cRBCs required for even a single transfusion unit (Table 2).

CLINICAL TRIALS

The first human transfusion of cRBCs was reported in 2011. HSCs were isolated from autologous mobilized PB, and 61,500±7,600-fold expansion was achieved by co-culture with mesenchymal stem cells. Subsequently, 10^{10} cRBCs were transfused and the half-life of the infused cells was approximately 26 days, demonstrating the safety of the cRBCs [39]. In 2022, a randomized and controlled phase I crossover trial titled RESTORE was announced to recruit at least ten healthy volunteers. Approximately 5–10 mL of lab-grown RBCs from allogeneic donors were transfused into 2 healthy individuals and no side effects have been reported. The details of current clinical trials of cultured red blood cells are shown in Table 3; ongoing studies will test the lifespan of lab-grown cells in the body and the donor's fresh blood.

Table 3. Clinical trials of cultured red blood cells.

Types	cRBC	
Cell origin	Autologous PB stem cells	Allogeneic PB stem cells
Infused cells	RBC	RBC
Recipient	Healthy volunteer	Healthy volunteers
Enrollment no.	1	2
Identifiers	NCT00929266	ISRCTN:42886452 EudraCT:2017-002178-38
Location	France	UK

CONCLUSIONS

Significant progress has been made in the generation of *in vitro* RBCs derived from immortalized cell lines and iPSCs and the development of the manufacturing process. Although product safety should be addressed further, pioneering clinical trials on cRBCs could herald a new era of transfusion medicine.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Stein DM, Upperman JS, Livingston DH, et al. National blood shortage: a call to action from the trauma community. J Trauma Acute Care Surg 2022;93:e119-22.
2. McGann PT, Weyand AC. Lessons learned from the COVID-19 pandemic blood supply crisis. J Hosp Med 2022;17:574-6.
3. An HH, Poncz M, Chou ST. Induced pluripotent stem cell-derived red blood cells, megakaryocytes, and platelets: progress and challenges. Curr Stem Cell Rep 2018;4:310-7.
4. Trakarnsanga K, Griffiths RE, Wilson MC, et al. An immortalized adult human erythroid line facilitates sustainable and scalable generation of functional red cells. Nat Commun 2017;8:14750.
5. Severn CE, Toye AM. The challenge of growing enough reticulocytes for transfusion. ISBT Science Series 2018;13:80-6.
6. Mei Y, Liu Y, Ji P. Understanding terminal erythropoiesis: an update on chromatin condensation, enucleation, and reticulocyte maturation. Blood Rev 2021;46:100740.
7. Migliaccio AR, Grazzini G, Hillyer CD. Ex vivo generated red cells as transfusion products. Stem Cells Int 2012;2012:615412.
8. Cannon M, Phillips H, Smith S, et al. Red blood cells differentiated in vitro using sequential liquid and semi-solid culture as a pre-clinical model. Exp Hematol Oncol 2021;10:50.
9. Seo Y, Shin KH, Kim HH, Kim HS. Current advances in red blood cell generation using stem cells from diverse sources. Stem Cells Int 2019;2019:9281329.
10. Heshusius S, Heideveld E, Burger P, et al. Large-scale in vitro production of red blood cells from human peripheral blood mononuclear cells. Blood Adv 2019;3:3337-50.
11. Bouhassira EE. Concise review: production of cultured red blood cells from stem cells. Stem Cells Transl Med 2012;1:927-33.
12. Sivalingam J, SuE Y, Lim ZR, et al. A scalable suspension platform for generating high-density cultures of universal red blood cells from human induced pluripotent stem cells. Stem Cell Rep 2021;16:182-97.
13. Xie X, Yao H, Han X, Yue W, Pei X. Therapeutic use of red blood cells and platelets derived from human cord blood stem cells. Stem Cells Transl Med 2021;10(Suppl 2):S48-53.
14. Kim S, Baek E. Cell sources for large-scale manufacture of red blood cells. ISBT Science Series 2018;13:268-73.
15. Satchwell TJ. Generation of red blood cells from stem cells: achievements, opportunities and perspectives for malaria research. Front Cell Infect Microbiol 2022;12:1039520.
16. Severn CE, Macedo H, Eagle MJ, Rooney P, Mantalaris A, Toye AM. Polyurethane scaffolds seeded with CD34+ cells maintain early stem cells whilst also facilitating prolonged egress of haematopoietic progenitors. Sci Rep 2016;6:32149.
17. Severn CE, Eissa AM, Langford CR, et al. Ex vivo culture of adult CD34+ stem cells using functional highly porous polymer scaffolds to establish biomimicry of the bone marrow niche. Biomaterials 2019;225:119533.
18. Lee E, Han SY, Choi HS, Chun B, Hwang B, Baek EJ. Red blood cell generation by three-dimensional aggregate cultivation of late erythroblasts. Tissue Eng Part A 2015;21:817-28.
19. Housler GJ, Miki T, Schmelzer E, et al. Compartmental hollow fiber capillary membrane-based bioreactor technology for in vitro studies on red blood cell lineage direction of hematopoietic stem cells. Tissue Eng Part C Methods 2012;18:133-42.
20. Allenby MC, Panoskaltsis N, Tahlawi A, Dos Santos SB, Mantalaris A. Dynamic human erythropoiesis in a three-dimensional perfusion bone marrow biomimicry. Biomaterials 2019;188:24-37.
21. Allenby MC, Okutsu N, Brailey K, et al. A spatiotemporal microenvironment model to improve design of a three-dimensional bioreactor for red cell production. Tissue Eng Part A 2022;28:38-53.
22. Mohebbi-Kalhori D, Behzadmehr A, Doillon CJ, Hadjizadeh A. Computational modeling of adherent cell growth in a hollow-fiber membrane bioreactor for large-scale 3-D bone tissue engineering. J Artif Organs 2012;15:250-65.
23. Phillips D, Shivaprasad P, Ellis M, Moise S. Design and optimisation of a novel fluidised bed bioreactor for the expansion of erythroid progenitor cells. Tissue Eng Part A 2022;28:S100.
24. Moise S, Pellegrin S, Frayne J, Toye AM, Ellis M. Fluidized bed bioreactor for the potential scale-up of an adult erythroid cell line. Nature Engineering Biology for Medicine 2019.
25. Boehm D, Murphy WG, Al-Rubeai M. The effect of mild agitation on in vitro erythroid development. J Immunol Methods 2010;360:20-9.
26. Timmins NE, Athanasas S, Gunther M, Buntine P, Nielsen LK. Ultra-high-yield manufacture of red blood cells from hematopoietic stem cells. Tissue Eng Part C Methods 2011;17:1131-7.
27. Griffiths RE, Kupzig S, Cogan N, et al. Maturing reticulocytes internalize plasma membrane in glycophorin A-containing vesicles that fuse with autophagosomes before exocytosis. Blood 2012;119:6296-306.
28. Kupzig S, Parsons SF, Curnow E, Anstee DJ, Blair A. Superior survival of ex vivo cultured human reticulocytes following transfusion into mice. Haematologica 2017;102:476-83.
29. Yu S, Vassilev S, Lim ZR, et al. Selection of O-negative induced pluripotent stem cell clones for high-density red blood cell production in a scalable perfusion bioreactor system. Cell Prolif 2022;55:e13218.
30. Zhang Y, Wang C, Wang L, et al. Large-scale ex vivo generation of human red blood cells from cord blood CD34+ cells. Stem Cells Transl Med 2017;6:1698-709.

31. dos Santos FF, Andrade PZ, da Silva CL, Cabral JM. Bioreactor design for clinical-grade expansion of stem cells. Biotechnol J 2013;8:644-54.
32. Zhang Y, Wang X, Pong M, Chen L, Ye Z. Application of bioreactor in stem cell culture. J Biomed Sci Eng 2017;10:485-99.
33. Ratcliffe E, Glen KE, Workman VL, Stacey AJ, Thomas RJ. A novel automated bioreactor for scalable process optimisation of haematopoietic stem cell culture. J Biotechnol 2012;161:387-90.
34. Glen KE, Workman VL, Ahmed F, Ratcliffe E, Stacey AJ, Thomas RJ. Production of erythrocytes from directly isolated or Delta1 Notch ligand expanded CD34+ hematopoietic progenitor cells: process characterization, monitoring and implications for manufacture. Cytotherapy 2013;15:1106-17.
35. Bayley R, Ahmed F, Glen K, McCall M, Stacey A, Thomas R. The productivity limit of manufacturing blood cell therapy in scalable stirred bioreactors. J Tissue Eng Regen Med 2018;12:e368-78.
36. Lee E, Lim ZR, Chen HY, et al. Defined serum-free medium for bioreactor culture of an immortalized human erythroblast cell Line. Biotechnol J 2018;13:e1700567.
37. Han SY, Lee EM, Lee J, et al. Red cell manufacturing using parallel stirred-tank bioreactors at the final stages of differentiation enhances reticulocyte maturation. Biotechnol Bioeng 2021;118:1763-78.
38. Gallego-Murillo JS, Iacono G, van der Wielen LAM, van den Akker E, von Lindern M, Wahl SA. Expansion and differentiation of ex vivo cultured erythroblasts in scalable stirred bioreactors. Biotechnol Bioeng 2022;119:3096-116.
39. Giarratana MC, Rouard H, Dumont A, et al. Proof of principle for transfusion of in vitro-generated red blood cells. Blood 2011;118:5071-9.