Received: 24 July 2020 | Accepted: 12 November 2020

DOI: 10.1111/jth.15181

REVIEW ARTICLE

Platelet production using adipose-derived mesenchymal stem cells: Mechanistic studies and clinical application

Yukako Ono-Uruga[1] | **Yasuo Ikeda**[2,3] | **Yumiko Matsubara**[1,4]

[1]Clinical and Translational Research Center, Keio University School of Medicine, Tokyo, Japan

[2]Department of Hematology, Keio University School of Medicine, Tokyo, Japan

[3]Life Science and Medical Bioscience, Waseda University, Tokyo, Japan

[4]Department of Laboratory Medicine, Keio University School of Medicine, Tokyo, Japan

Correspondence
Yumiko Matsubara, Clinical and Translational Research Center, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.
Email: yumikoma@keio.jp

Funding information
Grants-in Aid from Japan Agency for Medical Research and Development

Abstract

Megakaryocytes (MKs) are platelet progenitor stem cells found in the bone marrow. Platelets obtained from blood draws can be used for therapeutic applications, especially platelet transfusion. The needs for platelet transfusions for clinical situation is increasing, due in part to the growing number of patients undergoing chemotherapy. Platelets obtained from donors, however, have the disadvantages of a limited storage lifespan and the risk of donor-related infection. Extensive effort has therefore been directed at manufacturing platelets ex vivo. Here, we review ex vivo technologies for MK development, focusing on human adipose tissue-derived mesenchymal stem/stromal cell line (ASCL)-based strategies and their potential clinical application. Bone marrow and adipose tissues contain mesenchymal stem/stromal cells that have an ability to differentiate into MKs, which release platelets. Taking advantage of this mechanism, we developed a donor-independent system for manufacturing platelets for clinical application using ASCL established from adipose-derived mesenchymal stem/stromal cells (ASCs). Culture of ASCs with endogenous thrombopoietin and its receptor c-MPL, and endogenous genes such as *p45NF-E2* leads to MK differentiation and subsequent platelet production. ASCs compose heterogeneous cells, however, and are not suitable for clinical application. Thus, we established ASCLs, which expand into a more homogeneous population, and fulfill the criteria for mesenchymal stem cells set by the International Society for Cellular Therapy. Using our ASCL culture system with MK lineage induction medium without recombinant thrombopoietin led to peak production of platelets within 12 days, which may be sufficient for clinical application.

KEYWORDS

platelets, megakaryocytes, mesenchymal stem cells, platelet transfusion, adipose-tissue

Manuscript handled by: Matthew T. Rondina

Final decision: Matthew T. Rondina, 12 November 2020

1 | INTRODUCTION

Bone marrow cells called megakaryocytes (MKs) are large-sized cells with DNA polyploidy that form and release platelets during terminal thrombopoiesis.[1-3] Platelets have an essential role in the process of physiologic hemostasis. Thrombocytopenia, caused by an insufficient number of platelets, may be inherited or acquired, for example during chemotherapy.[4-6] Patients with severe thrombocytopenia often require platelet transfusions. The demand for platelet transfusions continues to increase globally. In both Europe and the United States more than 4.5 million donor-derived platelet units are transfused annually.[7] Donor-derived platelet concentrates have a

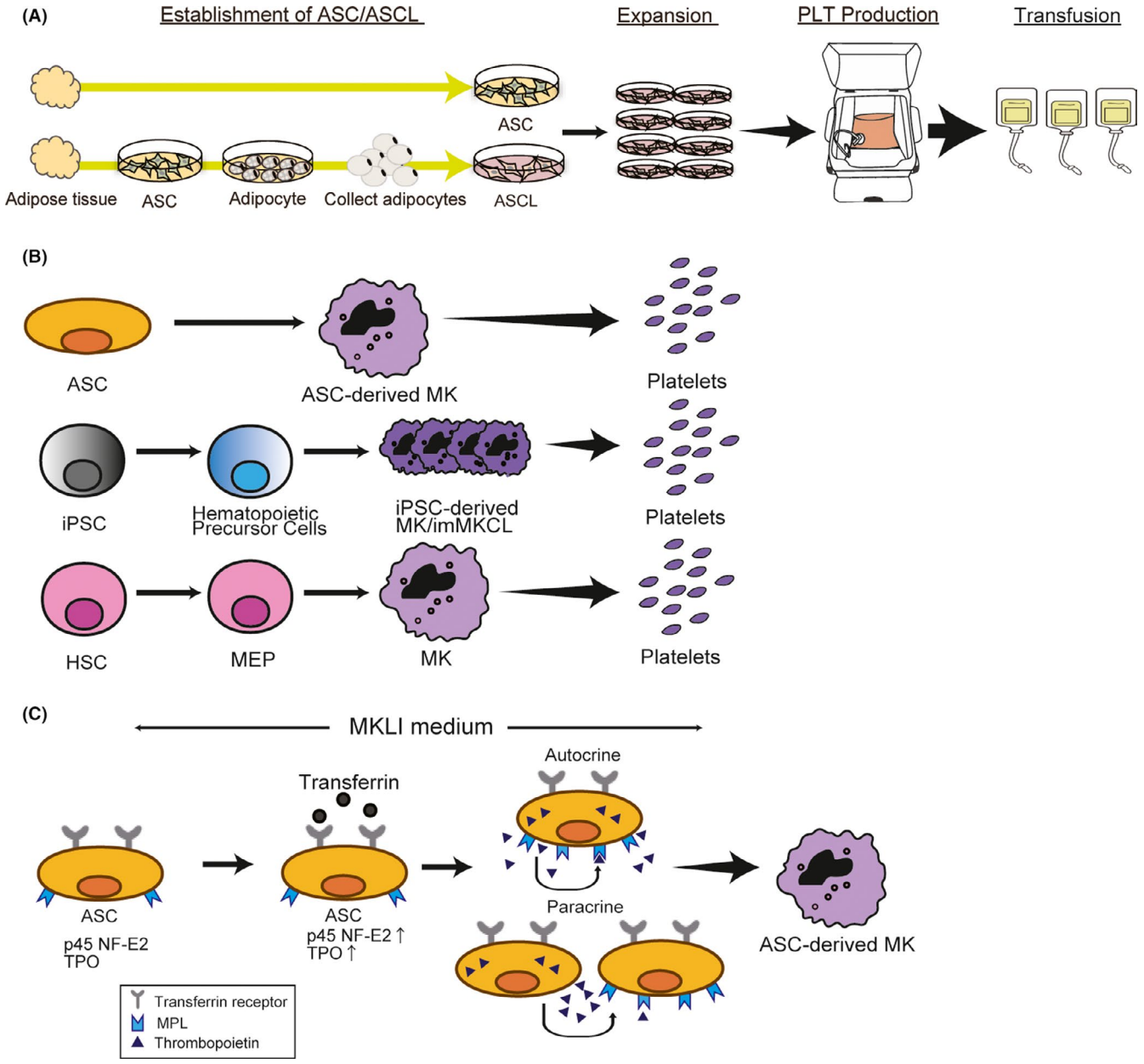

FIGURE 1 A, A schematic going from adipocytes to megakaryocytes (MKs) to platelets. There are two steps to obtain adipose-derived mesenchymal stem/stromal cell line (ASCL)-derived platelets. First, ASCL is established from adipose tissues by the upside-down method. When ASCL-derived platelets are needed, ASCL is cultured in megakaryocyte induction (MKLI) medium in the absence of recombinant thrombopoietin (TPO), described in the section "Production of MKs and platelets from MSCs/stromal cells," with a bioreactor. For the yield of ASCL-derived platelets, 1.5×10^{10} ASCLs were obtained from 10 g of adipose tissue, and ASCL release 1 to 10 platelets. Thus, 100 g of adipose-tissue is enough to obtain 10^{11} ASCL-derived platelets. ASC, adipose-derived mesenchymal stem/stromal cells; PLT, platelet. B, A schematic going from adipose-derived stem cells, iPSCs, and HSCs to MKs and platelets. Platelets reported to be differentiated from ASCs,[31] iPSCs,[14,24] and HSCs.[13] C, Endogenous TPO secreted via interactions between transferrin and its receptor, CD71, is involved in platelet differentiation from mesenchymal stem/stromal cells. Binding of transferrin to its receptor CD71 leads to the production of endogenous TPO. Interactions between TPO and its receptor c-MPL contribute to MK differentiation and subsequent platelet production. MKLI, MK lineage-induction medium in the absence of recombinant TPO

limited storage lifespan and carry a risk for bacterial infection. Novel approaches for supplying an adequate quantity of MKs and platelets will be needed to meet future demands.

Progress in technology has promoted regenerative medicine, and advanced ex vivo platelet production (Figure 1A,B).[8-25] Several kinds of stem cells, such as hematopoietic stem cells (HSCs), pluripotent stem cells, as well as endometrial stromal cells and nuclear factor, erythroid-derived 2 45KDa subunit *(p45NF-E2)*, *Maf K*-,and *Maf G*-transfected fibroblasts can be induced to differentiate into MKs and platelets by culturing them with MK induction medium and recombinant thrombopoietin (TPO).[11-25] Although MKs and platelets are easily produced from HSCs using this method, the proliferative abilities in vitro are unsatisfactory. Platelet yields are critical for establishing an ideal system for manufacturing platelets. More than 10^{11} platelets are used per transfusion in an adult patient. MKs proliferate little or not at all without gene transduction, and thus the development of an expandable MK cell source is critical for platelet manufacture. A system using induced pluripotent stem cells (iPSCs) has been most often used as starting cells to produce MKs and subsequent platelets. iPSCs have a proliferative activity in in vitro culture systems.

Several groups have developed a system for platelet manufacture using iPSCs,[12-25] and the technology for manufacturing MKs and subsequent platelets is rapidly developing. Table 1 shows recent reports of MK and platelet production. Nakamura et al reported how iPSCs need undergo additional gene manipulation for efficient amount of iPSC-derived MKs: to obtain the expandable MK cell line, immortalized megakaryocyte progenitor cell lines (imMKCL), iPSCs were transfected with BMI1 proto-oncogene, polycomb ring finger (*BMI1*) and *BCL2* like *(BCL-XL)* for the inhibition of cell senescence, and MYC proto-oncogene, bHLH transcription factor (*c-MYC*) for the promotion of cell proliferation.[14] One imMKCL released three to 10 platelets. Moreau et al generated long-term MKs after iPSCs were transfected with GATA binding protein 1 (*GATA1)* and Fli-1 proto-oncogene, ETS transcription factor*(Fli1)*, and TAL bHLH transcription factor 1, erythroid differentiation factor *(TAL1).*[21] They found that these MKs release five platelets per cell.

Within the bone marrow environment, platelets released from MKs are regulated by various factors of bone marrow niche, such as blood flow and extracellular matrix. Bioreactor for manufacturing platelets was developed based on bone marrow niche,[26] with two groups using their expandable MKs along with bioreactors to produce large number of platelets for clinical application (Table 1): Ito et al demonstrated that they can achieve 70 to 80 platelets from imMKCLs[14] using turbulence in a bioreactor.[24] Shepherd et al reported high platelet release of 30 platelets per MK using long-term MKs and a structurally graded collagen scaffold within a flow bioreactor system.[25] Moreover, iPSCs offer the ability to manipulate the nature of the resultant iPSC-derived MKs that can be of benefit in the transfusion of challenging patients. These include a targeted genetic manipulation to obtain human leukocyte antigen-lacking platelets and platelets with recipient-specific HPA-2.[12]

As an alternative to iPSC as a starting cell source, we demonstrated that MKs and platelets could be produced from mesenchymal cells isolated from adipose and bone marrow tissues.[27-32] Mesenchymal cells, which comprise mesenchymal stem cells (MSCs), preadipocytes, and stromal cells, can be obtained by centrifuging bone marrow or adipose tissues. Large amounts of adipose tissue are readily available. The stromal vascular fraction of mesenchymal cells is difficult to assess because of the nonhomogeneous nature of isolated cell fractions leading to the implantation of endothelial cells as well as different immune cell types.[33] We attempted to elucidate the mechanisms by which MKs and platelets are produced from mesenchymal cells and to identify the cell population responsible for their differentiation. We initially used commercially available preadipocytes. We then established a human adipose tissue-derived mesenchymal stem/stromal cell line (ASCL, Figure 1A, Table 1), and characterized these cells as MSCs according to the definition of the International Society for Cellular Therapy (ISCT).[33,34] A large-scale platelet manufacturing strategy has also been developed to be used with subcutaneous adipose-derived mesenchymal cells as cell source for platelet differentiation. Next, we focus on the advantages and limitations of the use of this line to produce human MKs and platelets, and their potential clinical application.

1.1 | Mesenchymal stem cells

Stem cells are able to regulate both self-replication and differentiate into many different cell types. Stem cells comprise at least two types, embryonic stem cells and adult stem cells. MSCs, a type of adult stem cell, are nonhematopoietic multipotent stem cells that can be collected from bone marrow, adipose tissue, umbilical cord, and dental pulp.[35] MSCs have a critical role in immunosuppression, inflammation resolution, and tissue repair.[36,37] The ISCT defined the minimal criteria for human MSCs according to three major characteristics as follows: the cells are adherent to plastic in culture with standard medium; most (>95%) of the cells express CD73, CD90, and CD105; and very few (<2%) of the cells express pan-leucocyte antigen CD45, hematopoietic and endothelial progenitor marker CD34, monocyte/macrophage antigen CD14 or CD11b, B lymphocyte antigen CD79α or CD19, or class II antigen human leukocyte antigen-DR. Finally, the cells must be capable of differentiating into adipocytes, osteoblasts, and chondrocytes.[33,34] Intensive studies aimed at establishing clinical applications for MSCs derived from bone marrow and adipose tissue have been performed.[38,39] The application of bone marrow-derived MSCs, as a regenerative medicine for patients with graft-versus-host disease, an immune-related disease, has been reported.[40]

1.2 | Production of MKs and platelets from MSCs/stromal cells

The molecular mechanisms underlying MK differentiation and platelet production are not fully understood. We previously studied a mechanism of platelet production using HSCs and

embryonic stem cells in vitro using OP9, a mouse bone marrow stroma cell line, as a feeder cell.[28,41] OP9 cells were established from macrophage colony-stimulating factor-deficient osteopetrotic mice.[41] OP9 cells have different cell lineages from hematopoietic cells and are reported to be preadipocytes and MSCs.[42] They are commonly used as feeder cells for the differentiation of stem cells, including embryonic stem cells and iPSCs, into hematopoietic cells.[41,43,44] When using MK lineage-induction (MKLI) medium,[27] composed of Iscove's Modified Dulbecco's Medium supplemented with 2 mM L-glutamine, 100 U/mL penicillin G sodium, 0.1 mg/mL streptomycin sulfate, 0.5% bovine serum albumin, 4 μg/mL low-density lipoprotein cholesterol, 200 μg/mL iron-saturated transferrin, 10 μg/mL insulin, 50 μM 2-β-mercaptoethanol, 20 μM each nucleotide (ATP, UTP, GTP, and CTP), and 50 ng/mL recombinant TPO. This medium generates MKs and platelets from HSCs. We unexpectedly observed the production of MKs and platelets from OP9 cells when studied as a negative control for platelet differentiation from embryonic stem cells. In addition, MKs and platelets could be produced from human subcutaneous adipose tissue and the mouse preadipocyte cell line 3T3-L1.[29,30] Based on these observations, we aimed to elucidate the mechanisms underlying the production of MKs and platelets from mesenchymal cells.

1.3 | Direct reprogramming of MKs and platelets from fibroblasts transfected with the transcription factor p45NF-E2

The lineage commitment from HSCs is mainly regulated by transcription factors and cytokines. We first planned to identify the factors that determine MK and thrombopoiesis induction. We observed that MKs and platelets are produced from the preadipocyte cell line 3T3-L1, but not its parent cell line, fibroblast cell line 3T3.[30] To screen for factors involved in MK induction, we compared the gene expression profiles of 3T3 cells and 3T3-L1 cells. Gene expression levels were examined on the basis of quantitative RT-PCR threshold cycle values. mRNA expression of CCAAT enhancer binding protein alpha (*CEBP alpha*), an important factor for adipocyte differentiation, and *p45NF-E2* was detected in 3T3-L1 preadipocyte cells. Neither factor was detected in 3T3 fibroblast cells.[11] We next evaluated whether adult human dermal fibroblasts can be induced to differentiate into MKs and platelets by gene transduction of candidate MK-inducing factors. Fibroblasts transfected with the transcription factors *p45NF-E2* and *Maf G/K*, binding proteins to p45NF-E2, had surface markers specific to MK lineages and intracellular factors related to MK lineages. The platelets released from the induced MKs were functional.[11] p45NF-E2 is a tissue-restricted transcription factor, whereas MafG/K proteins are expressed in many types of cells and exists in complex with p45NF-E2 to form NF-E2. Thus, p45NF-E2 appears key to the formation of MKs and platelets from nonhematopoietic adult fibroblasts.[11]

1.4 | MSCs/stromal cells express p45NF-E2, a key determinant of MK and platelet production

We found that MSCs/stromal cells express *p45NF-E2*, and gene expression levels increased during MK differentiation from MSCs/stromal cells.[28,32] We also observed *p45NF-E2* gene expression in MKs,[28,32] but not in embryonic stem cells.[28] Further, OP9 cells transfected with *p45NF-E2* produced more MKs and platelets, suggesting an important role of p45NF-E2 in their production. MSC/stromal cells also express genes, such as *GATA2* and *Fli1*, involved in the production of MKs and platelets.[28,32] Thus, MSCs/stromal cells may not require gene transfer to induce differentiation into MKs and platelets.

Additionally, we examined the gene expressions levels of several factors related to MKs and pluripotent stem cells using OP9 cells, mouse bone marrow mononuclear cells, and mouse embryonic stem cells.[28] Similar gene expression levels of *p45NF-E2* were observed in bone marrow mononuclear cells on days 0 and 4 of culture with MKLI medium. On the other hand, we observed increased expression of the *p45NF-E2* gene in OP9 cells on days 0, 4, and 8 of culture with MKLI medium.[28] Gene expression of Zinc finger protein, FOG family member 1 (*ZFPM1*, also called *FOG1)*, *Fli1*, *GATA2*. RUNX family transcription factor 1 *(RUNX1)*, and MPL proto oncogene, thrombopoietin receptor *(MPL)*, all involved in MK differentiation,[1-3,9,28] was observed in both bone marrow mononuclear cells and OP9 cells, and *GATA1* gene expression was detected in bone marrow mononuclear cells, but not in OP9 cells (as described in the following section). Increased expression of the *TPO* gene was detected in OP9 cells on days 0, 4, and 8 of culture with MKLI medium, but not in bone marrow mononuclear cells on days 0 and 4 of culture with MKLI medium.[28] Gene expression of SRY-box transcription factor 2 *(SOX2)*, related to pluripotent cells, was observed in mouse embryonic stem cells, but not in bone marrow mononuclear cells or OP9 cells.[28]

1.5 | Endogenous TPO secretion can induce the differentiation of MSCs into MKs and platelets

Cytokines and transcription factors have essential roles in the determining cell fate for MK lineages. TPO is an important cytokine that regulates the lineage commitment of MKs.[45-49] Recombinant TPO is commonly used in culture systems to induce a MK-rich population from stem cells, including HSCs, embryonic stem cells, and iPSCs. TPO production by the liver has been reported.[45,49] Although TPO production from MSCs/stromal cells is less well known, we found that OP9 cells and 3T3-L1 cells express *TPO* message.[28] Although *TPO* gene expression level increased during MK differentiation from these MSC/stromal cells, there was little TPO secretion from these cells cultured in maintenance media so that MK differentiation normally depends on endogenous TPO.[45-49]

We, therefore, investigated whether TPO endogenously released from MKs is sufficient to induce the differentiation of human

TABLE 1 Recent manufactured platelets

Starting Cells (Cell Source) for MK and Platelet Production			Additional Technology	
Cell Source	**Advantage**	**Disadvantage**	**Gene Transfer**	**Bioreactor**
CD34-positive cell (HSC)	Facile protocol to produce MKs and platelets	Difficult to obtain a sufficient number of HSCs from bone marrow, peripheral blood, and cord blood Low availability of proliferation in vitro	Not required	
iPSC	Higher proliferative activity in vitro	Requirement of sophisticated experimental techniques		
iPSC	Higher proliferative activity in vitro	Requirement of sophisticated experimental techniques Many procedural elements in the differentiation system	iPSC was transfected with BMI1, BCL-XL, and c-MYC for obtaining imMKCL	VerMES bioreactor with turbulent energy
iPSC	Higher proliferative activity in vitro	Requirement of sophisticated experimental techniques Many procedural elements in the differentiation system	iPSC was transfected with GATA1, FLI1, TAL1 for obtaining LT-fok MK	
iPSC	Higher proliferative activity in vitro	Requirement of sophisticated experimental techniques Many procedural elements in the differentiation system	iPSC was transfected with GATA1, FLI1, TAL1 for obtaining LT-fok MK	Structurally graded collagen scaffold within a flow Bioreactor system
Adipose-derived mesenchymal stem cell line (ASCL)	Higher proliferative activity in vitro More homogeneous population No abnormality of karyotype analysis after 60 days culture Facile protocol to produce MKs and platelets No need the addition of recombinant TPO	As compared with the imNKCL and LT-fokMK using a bioreactor (as previously), released number of platelets is lower in ASCL-MK than those iPS-derived MKs	Not required	Bioreactor with wave (Xuri)

adipose-derived mesenchymal stem/stromal cells (ASCs) into MKs and subsequent platelet production by culturing ASCs cultured in MKLI medium in the absence of recombinant TPO. TPO was secreted into the supernatant during MK differentiation from ASCs using MKLI medium in the absence of recombinant TPO, but was not detected in the supernatant from ASCs cultured in ASC maintenance

Yield (Production)			Platelet Function		
MK Production	Platelet Production	Surface Markers	Functional Test	Observations	Reference
10-20 MKs per CD34-positive cell					13
6-9 × 10^3 MKs per iPSC		27% (CD41/42b) of MKs			
10^6 to 10^{10} MKs for 15 days	70-80 platelets per MK (imMKCL)	72%-95.1% (CD41/42b) of platelets	Aggregation (collagen + ADP)	Similar data to donor platelets	24
			PAC-1 binding upon stimulation		
			P-selectin exposure upon stimulation		
			Bleeding time	Similar data to donor platelets	
2 × 10^5 MKs per iPSC for 90 days	5 platelets per LT-fokMK	68%-89% (CD41/42b) of MKs	perfusion	The fopMK platelets showed a shorter half-life than donor platelets	21
			Aggregation (ADP + TRAP)	Similar data to blood platelets	
			In vitro/in vivo thrombus formation	Similar data to blood platelets	
			Adhesion	Higher adhesion (%) than blood platelets	
			Spreading	Similar data to blood platelets	
	30 platelets per LT-fokMK	29.2 ± 15 CD41/42b platelets per CD41/42b MK	Fibrinogen binding upon stimulation	Similar data to donor platelets	25
			P-selectin exposure upon stimulation	37% exposure in Bioreactor platelets, 85% exposure in donor platelets	
			In vitro thrombus formation under flow	Similar data to donor platelets	
			Spreading	Bioreactor platelets show the typical stress fibers distribution	
	1-10 platelets per ASCL From 10 g adipose-tissue to 1.5 × 10^{10} ASCLs	1-10 CD42b platelets per ASCL	Fibrinogen binding upon stimulation	Similar data to platelet concentrate	32
			P-selectin exposure upon stimulation	Higher baseline than platelet concentrates	
			PAC-1 binding upon stimulation	Similar data to platelet concentrate	
			Spreading	Similar to previous data of donor platelets	
			Aggregation (collagen, ADP, Ristocetin, epinephrine)	Similar data to platelet concentrate	
			Infusion study for kinetics and incorporation into thrombus formation under flow	Similar data to platelet concentrate	

medium.[31] In contrast, we did not observe TPO in the supernatant obtained after MK differentiation from human HSCs (CD34-positive cells) cultured in the absence of recombinant TPO. We observed the production of MKs and platelets from ASCs cultured in MKLI medium in the absence of recombinant TPO, and this supernatant induced the differentiation of human HSCs (CD34-positive cells)

into MKs and platelets. These findings indicated that the endogenous TPO was sufficient to drive MK differentiation. MK differentiation was not observed in the cultures containing antibodies against the TPO receptor c-MPL,[31] supporting that released TPO stimulates ASCs to differentiate into MKs.

We investigated the mechanism of TPO secretion from ASCs during MK differentiation. Various components of the MKLI medium were examined to analyze the factor(s) responsible for TPO production. ASCs were cultured in each of the components of the MKLI medium in the absence of recombinant TPO. TPO secretion was only observed in the supernatant from cells cultured with transferrin-containing media.[31] On the basis of this screening test, we examined the effect of the transferrin receptor CD71 on TPO secretion. Using the small interference RNA-CD71, an anti-CD71 antibody, and CD71-positive/negative cells isolated by fluorescence-activated cell sorting, we explored the role of CD71 in the secretion of TPO from ASCs cultured with transferrin-containing MKLI medium in the absence of recombinant TPO. We observed that TPO was secreted in a transferrin dose-dependent manner. The TPO secretion levels and number of MKs produced by CD71-positive ASCs were significantly higher than those produced by CD71-negative ASCs. Thus, endogenous TPO binds to its receptor c-MPL on ASCs, which is involved in the production of ASC-derived MKs and platelets.[31] Interactions between transferrin and CD71 induce the secretion of endogenous TPO during MK differentiation from ASCs (Figure 1C). These finding provide novel insights into how ASCs differentiate into MKs and platelets. We also suggest the existence of a TPO-independent mechanism because MKs were also derived from CD71-negative ASCs.[31] MK production from CD71-negative ASCs was observed in the presence of anti-c-MPL antibody. The frequency of MKs from CD71-negative ASCs was lower than that from CD71-positive ASCs,[31] supporting that there might be a TPO-independent mechanism for MK production from ASCs.

1.6 | Development of a system for large-scale platelet production from MSCs

ASCs are candidate starting cells for manufacturing platelets for clinical application. Gene transfer is not necessary for inducing the differentiation of ASCs into MKs and platelets. The application of endogenous TPO promotes the differentiation of ASCs into platelets. Although ASCs are advantageous for manufacturing platelets, they are heterogeneous and may not be an efficient source of donor-independent platelets. We therefore established human ASCL as a cell source for large-scale platelet production (Figure 1A, Table 1). These cells meet the ISCT criteria for MSCs.[33,34] For the proliferation activity of ASCL, cells proliferate fast in the first 30 days, and reach a plateau after 60 days of culture.[32] To analyze the genetic karyotype stability, the ASCL were cultured for at least 60 days, and no abnormalities was observed in the ASCL.[32]

To characterize the differentiated cells, we performed flow cytometric analysis (CD41 and CD42b expression, and DNA ploidy) and immunostaining (serotonin, von Willebrand factor, platelet factor 4, and CD42b). The production of ASCL-derived MKs (ASCL-MKs) peaked on culture day 8. Evaluation of the gene expression associated with MK differentiation by quantitative RT-PCR revealed increased expression of Tublin beta class 1 (*beta 1 tubulin*), von Willebrand factor, *GATA2*, *TPO*, and *p45NF-E2*. Expression of genes related to MK and platelet production (*MPL*, platelet factor 4, *FOG1*, *Meis*, and *Fli-1*) was also detected during culture. We did not detect *GATA1* on culture days 0 (ASCL), 2 (immature MKs), 4 (immature MKs), and 6 (toward mature MKs from immature MKs). *GATA1* drives terminal erythroid development.[50] A previous report demonstrated that *GATA1*-deficient and *GATA1*-mutant cells undergo terminal differentiation into MKs coordinated by *GATA2*.[51]

We proposed that the ASCL would be an alternative source of ex vivo-generated MKs as the ASCL-MKs show significant numbers of overlapping genes with HSC-MKs. We therefore performed a microarray analysis on MKs derived from HSCs and the ASCL, and found that ASCL-MKs expressed specific MK lineage markers but also expressed high levels of the expression of mesenchymal markers such as Thy-1 cell surface antigen (*CD90*).[32]

To develop a system for the large-scale manufacture of ASCL-derived platelets, we used a bioreactor, a Xuri Cell Expansion System (GE Healthcare Life Sciences, Chicago, IL) containing ASCLs grown in 10 L of MKLI media in the absence of recombinant TPO (Figure 1A). Flow cytometric analysis and immunostaining were performed to characterize the ASCL platelets. From 10 L culture of 1×10^8 ASCLs, we obtained 1×10^8 CD42b-positive platelet-sized cell fragments after 4 to 20 days, and the production of ASCL-platelet peaked on culture day 12.[32] We next examined surface expression of MSC markers on the released ASCL platelets. The ASCL platelets differed from donor-derived platelets by expressing higher levels of CD10, CD13, CD44, CD73, CD90, and CD107b. For ASCL and platelet yield from ASCL, ASCLs have an advantage as a starting cell for manufacturing platelets with higher proliferative activity in vitro, relatively homogenous population, genetic stability (no abnormality of karyotype analysis after 60 days culture), and a simple protocol (Table 1). As compared with the imMKCL and LT-fokMK using a bioreactor (as described previously), released number of platelets is lower in ASCL-MKs than those iPS-derived MKs (Table 1). Thus, the period of ASCL expansion would need to be longer than iPSC/imMKCL and LT-fokMK to obtain similar number of ASCL platelets with imMKCL-derived platelets and LT-fokMK-derived platelets.

Platelet function studies were performed on ASCL platelets compared to donor-derived platelet concentrates obtained from the Japanese Red Cross. The ASCL platelets and platelet concentrates exhibited no spontaneous aggregation in the resting stage. Application of various agonists (eg, ADP, collagen, ristocetin, epinephrine) induced the aggregation of the ASCL platelets and platelet concentrates.[32] We examined PAC-1 binding and also P-selectin surface exposure, both markers of platelet activation,

on the two sources of platelets in similar agonist stimulation studies. Although baseline PAC1 binding and surface exposure of P-selectin tended to be higher on ASCL platelets than on platelet concentrates, both increased more on the ASCL platelets, supporting potential clinical utility upon transfusion. These (higher) functions might be partially associated with CD90 expression on ASCL platelets,[32] as described previously, and CD90 is involved in cell adhesion. Platelet adhesion has a critical role in the function. On the other hand, one possible way to reduce the hyperactivity of the ASCL platelets for infusion may be to decrease the number of infused cells. We are studying a monitoring system to optimize the use of ASCL platelets for infusion.

Also, we observed that the increased activation of ASCL platelets was regulated by aspirin. The thromboxane B_2 secretion levels under stimulation in ASCL platelets were lower than that in platelet concentrates, and the levels were decreased in the presence of aspirin, suggesting that baseline activity of ASCL platelets may be druggable.

Of importance is that, on transfusion into irradiation-induced thrombocytopenic, immunodeficient NOD/SCID gamma mice, the ASCL platelets and donor-derived platelets had similar half-lives and provided similar improved hemostatic function when assessed ex vivo in an automated microchip flow chamber system (Total Thrombus-formation Analysis System, Fujimori Kogyo Co., Ltd).[52,53] ASCL platelets collected on day 12 were used for the functional studies.[32] We do not have functional data of platelets collected on day 4 and day 20, and it is important to examine function of platelets collected on day 4 and day 20.

These studies also need to be repeated in a more clinically relevant model, perhaps using nonhuman primates, but suggest that the mild increased in baseline activity of ASCL platelets may be of limited clinical import.

In summary, we review the use of MSCs to generate MKs and platelets and describe their potential clinical use as well as compared MSC-derived MKs and platelets to donor- and iPSC-derived cells. The MSC cell line ASCL was established and characterized to develop a large-scale platelet manufacturing system. Gene transfer is not necessary for the establishing ASCLs or inducing their differentiation into ASCL-MKs and ASCL platelets under the influence of transferrin and the subsequent release of endogenous TPO by the ASCLs. Large-scale production of ASCL-MKs and ASCL platelets was achieved using a simple protocol. The resulting platelets, although having a mild increase in baseline platelet reactivity, were functional and had a normal half-life in murine models, increasing the feasibility of this approach for clinical application.

ACKNOWLEDGMENTS

The authors thank Drs. Kazuya Hosokawa and Tomoko Ohnishi (Research Institute, Fujimori Kogyo Co., Ltd) for critical advice in the perfusion study.

This work was supported by Grants-in Aid from Japan Agency for Medical Research and Development (Translational Research Network Program to YM).

CONFLICT OF INTEREST

Dr. Matsubara receives research support from Nissui Pharmaceutical, AdipoSeeds, and Fujimori Kogyo. Drs. Ono-Uruga, Ikeda, and Matsubara hold shares in AdipoSeeds and are inventors of a patent 6 425 308 (Japan), 10 113 147 (US), and PCT/JP2016/005016.

AUTHOR CONTRIBUTIONS

Yukako Ono-Uruga wrote the portion for platelets from mesenchymal stem cell line. Yasuo Ikeda wrote the portion for iPS-derived platelets. Yumiko Matsubara wrote and edited the manuscript , and supervised to prepare the manuscript.

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How to cite this article: Ono-Uruga Y, Ikeda Y, Matsubara Y. Platelet production using adipose-derived mesenchymal stem cells: Mechanistic studies and clinical application. *J Thromb Haemost*. 2020;19:342–350. https://doi.org/10.1111/jth.15181