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REVIEW ARTICLE

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Development of platelet replacement therapy using human induced pluripotent stem cells

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Abstract

In the body, platelets mainly work as a hemostatic agent, and the lack of platelets can cause serious bleeding. Induced pluripotent stem (iPS) cells potentially allow for a stable supply of platelets that are independent of donors and eliminate the risk of infection. However, a major challenge in iPS cell-based systems is producing the number of platelets required for a single transfusion (more than 200 billion in Japan). Thus, development in large-scale culturing technology is required. In previous studies, we generated a self-renewable, immortalized megakaryocyte cell line by transfecting iPS cell-derived hematopoietic progenitor cells with c-MYC, BMI1, and BCL-XL genes. Optimization of the culture conditions, including the discovery of a novel fluid-physical factor, turbulence, in the production of platelets in vivo, and the development of bioreactors that apply turbulence have enabled us to generate platelets of clinical quality and quantity. We have further generated platelets deleted of HLA class I expression by using genetic modification technology for patients suffering from alloimmune transfusion refractoriness, since these patients are underserved by current blood donation systems. In this review, we highlight current research and our recent work on iPS cell-derived platelet induction.

KEYWORDS
bioreactor, iPS cell, megakaryocyte, platelet, turbulence

1 | INTRODUCTION

Normal hematopoiesis in adults takes place in the bone marrow, which produces leukocytes, red blood cells, and platelets. On the other hand, fetal hematopoiesis takes place in different regions at different stages of development. There are two main stages of fetal hematopoiesis development: primitive hematopoiesis, which occurs transiently in the yolk sac in the early stage of the embryo, and definitive hematopoiesis, which begins after primary hematopoiesis and changes its location from the aorta-gonad-mesonephros (AGM) to the liver and then to the bone marrow. The blood system maintains and protects our body by performing three main functions: oxygen delivery, coagulation, and immunity. Platelets, which have clotting function, are released by megakaryocytes in the bone marrow and circulate as anucleate blood cells. Normal thrombocytosis consists of mature megakaryocytes elongating a cytoplasmic structure called the proplatelet. The tip of the proplatelet extends into the bone marrow sinus, where it is sheared by the bloodstream and becomes a platelet (Junt et al., 2007; Kosaki, 2008; Machlus & Italiano, 2013; Thon et al., 2010). Recently, it was reported that megakaryocytes are also present in the mouse lung and spleen and similarly release platelets by the force of blood flow (Lefrançais et al., 2017).

Blood donor-derived blood products are provided for the treatment of a variety of diseases and conditions that cause cytopenia (Estcourt et al., 2017). However, there is a need to strengthen the blood transfusion system because of decreasing blood donors due to low birthrates and changing attitudes of the young population, contamination by pathogens, and increasing demand due to chronic blood diseases, which are common in aged populations. Platelet preparations are suitable for storage at room temperature to maintain platelet function but only have a statutory expiration date of 5 days or less. Therefore, platelet preparations are difficult to stock. In addition, some patients bear alloantibodies against human leukocyte antigen class I (HLA-I) that are expressed on platelets, resulting in alloimmune platelet transfusion refractoriness (allo-PTR; Stanworth et al., 2015). Effective treatment requires HLA-compatible platelet transfusions, but supply shortages often leave patients underserved. Pluripotent stem cells, such as embryonic stem (ES) cells (Thomson, 1998) and induced pluripotent stem (iPS) cells (Takahashi et al., 2007), can proliferate indefinitely in vitro and are an attractive source for the development of blood transfusion products as an alternative to blood donations. Platelets are anucleate cells; thus, the risk of tumorigenesis due to iPS cell contamination can be avoided by irradiation prior to the blood transfusion. In addition, by applying stocked iPS cells with homozygous HLA, it is possible to complement the current donor-dependent blood transfusion system by providing a stable supply of HLA-compatible platelets. More recently, it has become possible to produce HLA class I-deleted blood products to avoid rejection by genetic modification techniques such as the CRISPR/Cas9 method (Cong et al., 2013; Mali et al., 2013). In this review, we focus on our recent developments in the ex vivo production of iPS cell-derived platelets, including the development of megakaryocyte cell lines, bioreactors, and scale-up cultures, and the discovery of viable drugs in manufacturing. We will also discuss the universal potential of HLA-null, iPSC-derived platelet products.

2 | DEVELOPMENT OF MEGAKARYOCYTE HEMATOPOIESIS

In the process of ontogeny, hematopoiesis begins with a process known as primitive hematopoiesis. Studies on hematopoietic development have been carried out for many years, mainly in mice. The first observed moment of hematopoiesis in the fetal period of mice is the formation of structures called blood islands in the yolk sac, an extrafetal membranous tissue that surrounds the fetus from about day 7 of the fetal period (Moore & Metcalf, 1970). In blood islands, fetal red blood cells having fetal hemoglobin are mainly produced (Silver & Palis, 1997), but so too are macrophages (Palis et al., 1999) and megakaryocytes (Tober et al., 2007). Definitive hematopoiesis begins with the development of hematopoietic stem cells (HSCs) from the AGM region in the fetus around day 10 of the fetal period, with the generated HSCs migrating to the liver (Medvinsky & Dzierzak, 1996).Thereafter, on day 16 of the fetal period, the HSCs repeatedly self-renew and differentiate (Ema & Nakauchi, 2000), simultaneously increasing themselves and a variety of mature blood cells. Immediately after birth, the fetal liver is the main place of hematopoiesis, but just before birth, HSCs shift to the bone marrow so that hematopoiesis also occurs there, and in adults is the place of hematopoiesis throughout life. HSCs can be divided into two types: long-term (LT) HSCs, which can be transplanted into mice with reduced hematopoietic capacity (Osawa et al., 1996), and short-term (ST) HSCs, which have pluripotency but only temporary self-renewal ability (Christensen & Weissman, 2001). As differentiation progresses from ST-HSCs, the cells differentiate into multipotent progenitors (MPPs), which have no ability to self-renew. MPPs are destined to be the progenitors of all blood cells Subsequently, progenitors, including common myeloid progenitors (CMP), granulocyte/monocyte progenitors (GMP), megakaryocyte/erythroblast progenitors (MEP), and common lymphoid progenitors (CLP) in the lymphoid system, have been purified and identified, and the pathways of their hematopoietic differentiation system have been clarified (Akashi et al., 2000; Kondo et al., 1997). The conventional model for megakaryopoiesis is to differentiate HSCs to MPPs, then CMPs, and MEPs, which are megakaryocyte progenitors. An important cytokine for megakaryocyte differentiation is thrombopoietin (TPO) (Bartley et al., 1994; Kaushansky et al., 1994), which is mainly produced by the liver. In addition, various cytokines such as interleukin (IL)-3 (Teramura et al., 1988), IL-6 (Navarro et al., 1991), IL-11 (Broudy et al., 1995), GM-CSF (Briddell et al., 1991), SCF (Briddell et al., 1991), and LIF (Metcalf et al., 1991) have been reported to promote megakaryocyte differentiation, but through supplementary actions. The TPO receptor, c-MPL, is a single transmembrane receptor that dimerizes when TPO binds. Subsequently, the phosphorylation of tyrosine residues on the c-MPL receptor leads to activation of the JAK2/STATs pathway and activation of MAPKs and PI3K/Akt by RAS, resulting in the expression of a group of genes involved in megakaryocyte differentiation (Beer et al., 2008; Drachman et al., 1995; Grozovsky et al., 2015). The transcription factors involved in megakaryocyte differentiation include GATA-1 and FLI-1, which are also involved in the regulation of *mpl* gene expression and differentiation into erythrocytes and megakaryocytes in MEP fractions (Deveaux et al., 1996; Frontelo et al., 2007; Stachura et al., 2006). During megakaryocyte maturation, FOG1/GATA1 complex, RUNX1, and FLI-1 have all been reported important for the transcriptional regulation of megakaryocyte differentiation (Lordier et al., 2012; Shimizu et al., 2004, 2009; Wang et al., 2002). NF-E2 promotes mature megakaryocytes to form proplatelets, thus contributing to platelet production, and also regulates platelet activity (Levin et al., 1999; Shivdasani et al., 1995). Although the above describes the classical model for megakaryocyte and platelet differentiation, recently, megakaryocyte repopulating progenitors (MKrPs) were found to exist in the HSC population (Yamamoto et al., 2013). MKrPs are destined to differentiate directly from HSCs into megakaryocytes. Additionally, it has been demonstrated that some HSCs expressing von Willebrand factor (vWF), which is expressed on megakaryocytes, are destined to differentiate directly into megakaryocytes (Sanjuan-Pla et al., 2013). MPPs have also been divided into four groups (MPP1-4), each of which has a

biased differentiation lineage. In particular, MPP2 is biased toward megakaryocyte and erythrocyte differentiation (Pietras et al., 2015). Indeed, it has also been reported that MPP2 differentiate directly into megakaryocytes (Rodriguez-Fraticelli et al., 2018). These reports suggest a new pathway that complements the conventional megakaryocyte differentiation model (Figure 1; Noetzli et al., 2019).

3 | ADVANTAGES OF iPS CELL-DERIVED BLOOD PRODUCTS

iPS cells have pluripotency and self-renewal capacity and can proliferate indefinitely in vitro. Reconstruction of the hematopoietic system using iPS cells would contribute to understanding the developmental mechanism. Furthermore, iPS cells established from specific patients can be used for disease modeling, contributing to understanding of the pathology and to drug screening. Since iPS cells can easily be genetically engineered using CRISPR/Cas9 and other methods, the role of specific genes can be addressed in the development and pathogenesis. For regenerative medicine, patients themselves and highly immunocompatible iPS cells could be the source cells. Based on previous studies using ES cells, the differentiation of iPS cells into various types of blood cells, such as lymphocytes (Nishimura et al., 2013; Themeli et al., 2013; Vizcardo et al., 2013), myeloid cells (Haruta et al., 2013), erythrocytes (Hirose et al., 2013; Kurita et al., 2013), and platelets, has been successfully achieved, and attempts for clinical applications are underway. The advantages

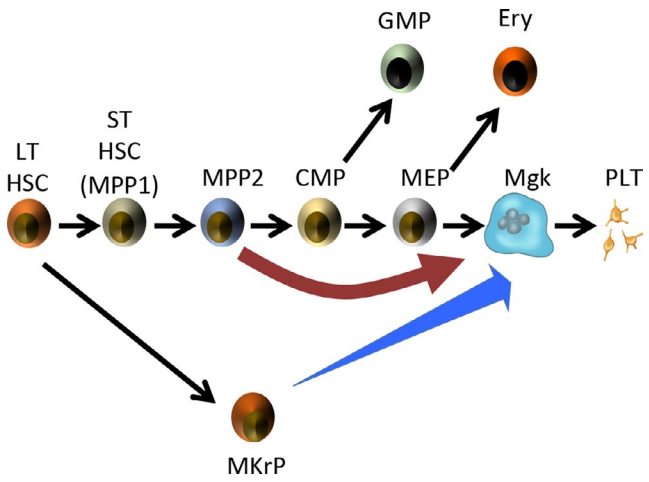

FIGURE 1 Model for megakaryocyte differentiation. Conventional hematopoietic differentiation involves the division of hematopoietic stem cells into lymphoid and myeloid progenitor cells, from which they are terminally differentiated into individual cells. Recently, it has been reported that megakaryocyte differentiation can be performed from LT-HSCs via MKrP and directly from MPP2. CMP, common myeloid progenitor; Ery: erythrocyte; GMP, granulocyte/macrophage progenitor; LT-HSC, long-term hematopoietic stem cell; MEP, megakaryocyte/erythroid progenitor; Mgk, megakaryocyte; MKrP, megakaryocyte repopulating progenitor; MPP2, multipotent progenitor 2; PLT, platelet; ST-HSC, short-term hematopoietic stem cell (or MPP1)

of iPS cell-derived blood products are that nonpathogenicity is guaranteed by sterile manufacturing from the source cells without the risk of infection and the supply is independent of blood donors. The establishment of various blood cell lines that can self-replicate and be cryopreserved from iPS cells would provide a large number of safe and functional blood cell lines that could be stocked as a master cell bank. By following good manufacturing practice or good gene, cellular, and tissue-based products manufacturing practice (GCTP) standards for the manufacturing process after thawing, it would be possible to repeatedly produce blood products of guaranteed clinical grade quality.

4 | DIFFERENTIATION OF PLATELETS USING ES AND iPS CELLS

In Japan, 10 units of platelets (about 200 billion platelets) are transfused in a single transfusion. By taking advantage of the indefinite self-renewal capacity of iPS cells, we and other groups have created a strategy to amplify large numbers of iPS cells from which we generate megakaryocytes and platelets. In our differentiation method, ES/iPS cell-derived sac-like structures (ES/iPS-sac) are induced by co-culturing mouse fetal 10T1/2 cells with ES/iPS cells in the presence of vascular endothelial growth factor (VEGF). Blood progenitor cells are contained in the sac. These progenitor cells can be directly differentiated into megakaryocytes and platelets by culturing them in the presence of soluble factors such as SCF and TPO (Takayama et al., 2008, 2010). The obtained platelets form thrombi in vivo. Feng et al. succeeded in differentiating iPS cells directly into megakaryocytes and platelets in a feeder-free, serum-free medium and animal component-free condition using the EB method (Feng et al., 2014). They also differentiated genetically engineered HLA-null iPS cells into megakaryocytes and platelets using the TALEN method and showed that the obtained platelets had functional capacity. Hansen et al. have succeeded in differentiating single iPS cells into blood progenitor cells using the monolayer differentiation system in feeder-free, serum-free condition and differentiating the progenitor cells into megakaryocyte, erythroid, and myeloid cells (Hansen et al., 2018). However, none of these methods have been developed for practical use due to the complexity of the operations and the long incubation processes required. As a solution to the above problems, we established new technology to immortalize megakaryocytes.

5 | ESTABLISHMENT OF iPS CELL-DERIVED IMMORTALIZED MEGAKARYOCYTE CELL LINES

Based on our previous ES/iPS cells differentiation system, we realized that the expression of c-MYC is upregulated during the proliferation phase of megakaryocyte progenitor cells but repressed during the maturation phase of megakaryocytes (Takayama et al., 2010). Accordingly, c-MYC transgenic mice have an increased

megakaryocyte population, although no significant change in platelet counts compared to wild type (WT; Thompson et al., 1996). On the other hand, c-MYC knockout mice show low-ploidy (<8N) megakaryocytes and high MPV platelets. In addition, both megakaryocyte and platelet counts are increased in c-MYC knockout mice compared with WT (Guo et al., 2009). These findings suggest that the controlled c-MYC expression may be important for proliferation and maturation in megakaryocyte development. The overexpression of c-MYC in ES cell-derived blood progenitors transiently increased the proliferation of megakaryocytes, followed by the induction of cellular senescence and apoptosis. Given that the polycomb complex component BMI1 and the BCL2 family member BCL-XL respectively repress the cellular senescence-inducing INK4A/ARF gene locus and apoptosis, we succeeded to establish immortalized megakaryocyte cell lines (imMKCLs) by sequentially transferring the c-MYC and BMI1 genes followed by the BCL-XL gene into human iPS/ES-derived hematopoietic progenitor cells (Nakamura et al., 2014). Since these three genes are regulated by the Tet-On system in our system, the addition of doxycycline enables imMKCLs to proliferate with the expression of the three genes turned on (DOX ON), whereas depletion of doxycycline suppresses their expression (DOX OFF) and induces imMKCL to mature and release platelets (Figure 2). By stocking large quantities of imMKCL strains as a master cell bank and confirming their safety and high productivity, we proposed a system to produce a large number of clinically applicable platelets from these imMKCL master cells by liquid culture in accordance with good manufacturing practice or GCTP clinical grade standards.

However, the high cost demands optimizing the culture media conditions. Expensive recombinant proteins such as stem cell factor (SCF) and thrombopoietin (TPO) were necessary in the original imMKCL medium, but we have since developed a TPO-like agonist, small molecule TA-316 (Aihara et al., 2017). We also developed KP-457, which inhibits a disintegrin and metalloprotease 17 (ADAM17) activity, which cleaves the extracellular surface of the vWF receptor GPIbα (CD42b) under culture conditions at 37°C (Hirata et al., 2017).

Meanwhile, Moreau et al. established a cryopreservable and expandable megakaryocyte cell line, forward programmed megakaryocytes (fopMKs), by forcefully expressing GATA1, FLI1, and TAL1 during iPS cell differentiation into megakaryocytes (Moreau et al., 2016). In a non-iPS cell-based approach, Ono et al. directly induced human and mouse fibroblasts into megakaryocytic cells by overexpressing NF-E2, Maf-G, and Maf-K (Ono et al., 2012). They also successfully induced megakaryocytes from a human adipose-derived mesenchymal stem cell line that proliferated for more than 2 months (Tozawa et al., 2019). These cell lines could also be a useful source if optimal large-scale culture conditions are achieved.

6 | DISCOVERY OF NOVEL PHYSICAL FACTORS IN IN VIVO PLATELET PRODUCTION

The culture of imMKCLs during the proliferation and maturation stages was feeder cell dependent (adhesion dependent), but a feeder cell-independent culture is essential for clinical application. During the growth phase, we successfully expanded imMKCLs sequentially in a 100-ml flask and a 1–20-L WAVE bag system with mild rocking motion without feeder cells. In the maturation phase, the screening of candidate drugs led to the discovery that the combination of Rho-associated protein kinase (ROCK) inhibitors (Gobbi et al., 2013) and aryl hydrocarbon receptor (AhR) antagonists (Strassel et al., 2016) enabled platelet production in feeder-free conditions (Ito et al., 2018).

Two-photon microscopy had shown that the cytoplasm extends from megakaryocytes in the bone marrow to the lumen of blood vessels, where the tips of the protrusions are cleaved by blood flow stimulation (Junt et al., 2007). It was proposed that this cytoplasmic

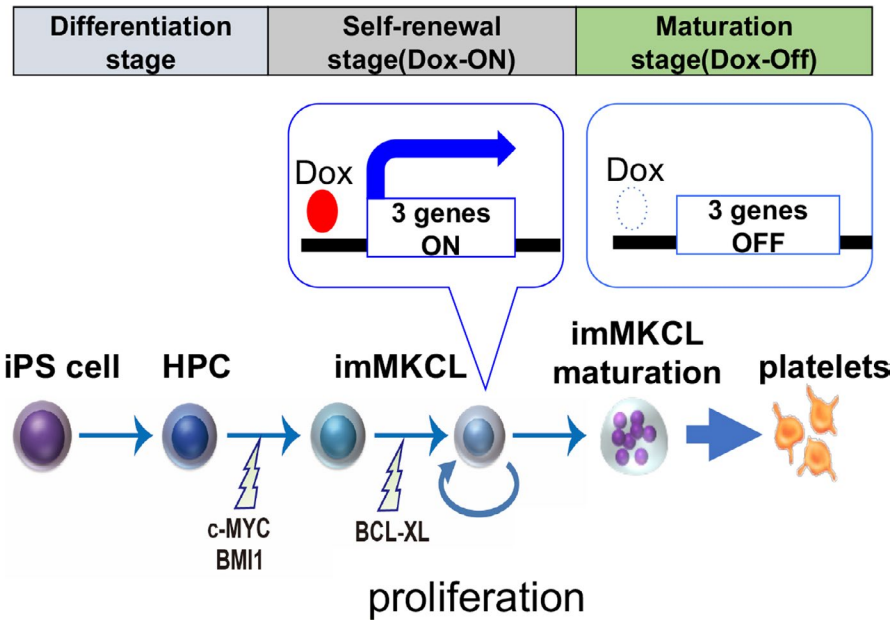

FIGURE 2 Self-renewal of and platelet production by immortalized megakaryocyte progenitor cell lines (imMKCL). By introducing c-MYC, BMI1, and BCL-XL into iPSC-derived hematopoietic progenitor cells, imMKCLs can be established. These genes are regulated by the Tet-on system. The cells proliferate with doxycycline (Dox) addition and mature and produce platelets with Dox removal (Nakamura et al., 2014)

cleavage is primarily caused by shear stress. Based on this concept of shear stress, various groups, including ours, have developed bioreactors (Avanzi et al., 2016; Di Buduo et al., 2015; Nakagawa et al., 2013). Thon et al. developed a microfluidic platelet bioreactor designed to mimic the bone marrow environment by loading human umbilical vein endothelial cells (HUVECs) and extracellular matrix components onto a chip (Thon et al., 2014). Blin et al. designed a bioreactor consisting of multiple vWF-coated micropillars that act as anchors for megakaryocytes to facilitate shear stress efficiently on megakaryocytes (Blin et al., 2016). However, because the platelet production efficiency was low for all reactors, we suspected that shear stress alone does not fully capture the bone marrow environment. Therefore, we applied two-photon microscopy and particle image velocimetry (PIV) to analyze platelet production sites in the bone marrow in more detail. We found that platelets were sheared and released from the proplatelets at a location in the bloodstream exposed to turbulence, suggesting that turbulence is also a physical factor in platelet production (Ito et al., 2018).

7 | PRODUCTION OF iPS CELL-DERIVED PLATELET PREPARATIONS

Based on the discovery that turbulence contributes to platelet generation in vivo, we cultured imMKCLs in a 2.4-L vertical reciprocal motion liquid culture bioreactor (VerMES) with controllable turbulent physical conditions. With this system, we succeeded in producing iPS cell-derived platelets that are comparable to the function of platelets in vivo, in a highly efficient manner (about 80 platelets per megakaryocytes). A simulation analysis of the physical parameters and platelet production in culture vessels under various conditions of turbulence were investigated using 0.3-L and 2.4-L VerMES. We found that the optimal values of shear stress and turbulent energy were independent of the volume of the VerMES culture vessel. Accordingly, we optimized shear stress and turbulent energy in an 8-L VerMES to generate 100 billion functional platelets. Electron microscopic observation showed that iPS cell-derived platelets and platelets in vivo had similar ultrastructures. In addition, evaluation in thrombocytopenic mouse and rabbit models (Watanabe et al., 2017) confirmed that the iPS cell-derived platelets had a hemostatic function similar to that of donor platelets (Ito et al., 2018).

Since a microarray analysis of imMKCLs cultured in VerMES and static cultures showed almost no difference in gene expression profiles, we hypothesized that critical factors that promote platelet generation may be released from imMKCLs in VerMES cultures (Ito et al., 2018). Proteomic analysis of the culture supernatant revealed an increase in the secretion of six proteins in the VerMES culture compared with the culture under static conditions. Interestingly, the addition of VerMES culture supernatant to the static culture had no effect on the platelet production, suggesting that soluble factors and physical stimuli, mainly shear stress, work together to contribute to the platelet production. In particular, nardilysin (NRDC; Nishi, 2013), macrophage migration inhibitory factor (MIF; Strüßmann et al., 2013), and insulin-like growth factor binding protein 2 (IGFBP2; Coppé et al., 2008) were shown to promote platelet production. Observations in a microfluidic chip environment revealed that proplatelet formation was significantly reduced in cultures without MIF or IGFBP2. Further analysis suggested that in the absence of MIF and IGFBP2, proplatelet formation was impaired due to reduced extracellular matrix secretion. NRDC was released in a turbulent microenvironment surrounding proplatelets and suggested to be involved in the fragmentation of the proplatelets by endopeptidase activity (Nishi, 2013). In summary, turbulent flow stimulation is suggested to create an environment for the formation of proplatelets through an autocrine mechanism that ultimately cleaves the cell membrane using shear stress to produce platelets (Figure 3).

8 | DIFFERENCES BETWEEN IN VITRO AND IN VIVO IN MEGAKARYOCYTE MATURATION AND PLATELET PRODUCTION

Adult megakaryocytes in the bone marrow take the form of giant polyploid cells with chromosome numbers ranging from 16N to 124N as they mature, migrate into vascular niche, come into contact with collagen type IV, which is present in the basement membrane of the vein, and form proplatelet protrusions to release platelets into the blood (Semeniak et al., 2016). In contrast, iPS cell-derived megakaryocytes are mainly poorly polyploid cells, ranging from 2N to 32N (Takayama et al., 2010). Similarly, megakaryocytes differentiated from peripheral blood-derived $CD34^+$ cells in vitro range from 2N to a maximum of 32N as well (Liu et al., 2011). The proplatelet formation in vitro occurs as protrusions at several points throughout the cell membrane, and these protrusions elongate to release platelets.

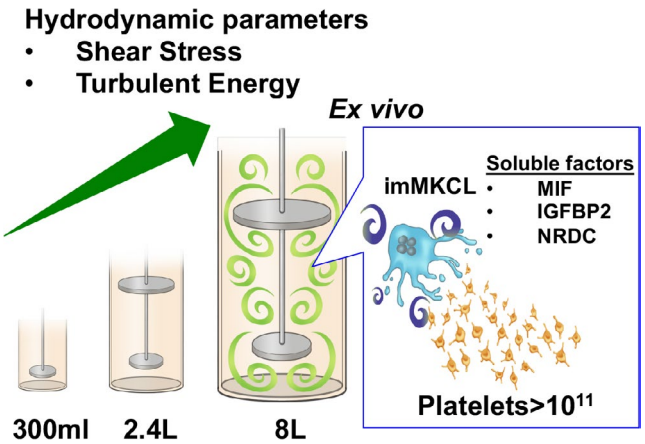

FIGURE 3 Culture for large-scale platelet production. Optimal values of turbulent energy and shear stress for platelet production enable scale-up of the VerMES culture. We achieved 10 billion functional platelets in 8-L VerMES through this scale-up. The soluble factors NRDC, IGFBP2, and MIF are released from imMKCLs in the turbulent environment to enhance platelet biogenesis (Ito et al., 2018)

A single MK in the bone marrow is estimated to produce 800–2,000 platelets, whereas imMKCLs had a lower platelet production of 60–80 platelets per cell. In addition, iPS-derived platelets are as large as 2–10 μm compared to 2–4 μm for platelets in vivo. Interestingly, the large iPS-derived platelets have been found to be fragmented by blood flow after administration in mice (Ito et al., 2018).

Currently, due to poor polyploid and platelet production in vitro compared with in vivo, it is assumed that the role of vascular niche and bone marrow environment has not been fully adapted to culture conditions in vitro.

9 | iPS CELL-DERIVED PLATELETS FOR HLA COMPATIBILITY

Gestation or repeated platelet transfusion can lead to sensitization and the production of antibodies against non-self HLA-I. As a consequence, allo-PTR, a rejection of transfused platelets due to incompatibility with mostly HLA-I, occurs in 5%–15% of patients receiving platelet transfusions. These patients require HLA-matched platelets, which may not be available for rare types or in emergencies (Stanworth et al., 2015). iPS cell-derived platelets can cope with allo-PTR in a few ways. First, autologous platelets can be produced by establishing iPS cells from the patient (https://jrct.niph.go.jp/en-latest-detail/jRCTa050190117). This product is by nature completely compatible including HLA and human platelet antigen (HPA), which is another alloantigen on platelets that can also cause allo-PTR and post-transfusion purpura, in which platelet counts become even lower post-transfusion (Semple et al., 2011; Stanworth et al., 2015). However, producing autologous platelets for each person takes considerable cost and time.

Alternatively, our institute and others have been stocking iPS cells with homozygous HLA haplotypes (Turner et al., 2013; Umekage et al., 2019). These cells have wide compatibility. It is estimated that the 10 most frequent lines of iPSCs with homozygous HLA could cover approximately 50% of the Japanese population. Ultimately, owing to the capability of gene editing iPS cells, HLA-I nullified iPS cell-derived platelets have also been developed (Feng et al., 2014; Gras et al., 2013; Suzuki et al., 2020) These cells can serve as a universal product and do not require a library of different HLA haplotypes. Furthermore, they are suitable as a platform for further modified products, which may lead to novel therapies using platelets.

10 | CONCLUSION

Thirteen years have passed since the report of the establishment of human iPS cells in 2007. Platelets derived from iPS cells have been successfully manufactured to produce the number needed for clinical transfusions. As such, the first-in-human clinical trial of autologous iPSC-derived platelets in patients with allo-PTR was initiated in 2019 (https://jrct.niph.go.jp/en-latest-detail/jRCTa050190117). One of the current issues is to reduce the cost per production. In this regard, it is necessary to further mature imMKCLs by constructing a culture system that mimics the in vivo environment. In the future, further innovations will achieve industrialized levels and open new medical systems that will enable safe and ready transfusion systems for any circumstance.

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CONFLICT OF INTERESTS

K.E. and S.N. submitted patents related to some references (Ito et al., 2018; Nakagawa et al., 2013; Nakamura et al., 2014; Nishimura et al., 2015; Takayama et al., 2008, 2010). K.E. is a cofounder of Megakaryon Corporation and a member of its scientific advisory board without salary. K.E. receives grants from Megakaryon, Otsuka Pharmaceutical Co., Ltd., and Kyoto Seisakusho Co., Ltd. The interests of K.E. were reviewed and are managed by Kyoto University in accordance with its conflict-of-interest policies. S.N. and N.S. declare no conflict of interest.

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