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Induction of differentiation of human stem cells *ex vivo*: Toward large-scale platelet production

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Abstract

Platelet transfusion is one of the most reliable strategies to cure patients suffering from thrombocytopenia or platelet dysfunction. With the increasing demand for transfusion, however, there is an undersupply of donors to provide the platelet source. Thus, scientists have sought to design methods for deriving clinical-scale platelets *ex vivo*. Although there has been considerable success *ex vivo* in the generation of transformative platelets produced by human stem cells (SCs), the platelet yields achieved using these strategies have not been adequate for clinical application. In this review, we provide an overview of the developmental process of megakaryocytes and the production of platelets *in vivo* and *ex vivo*, recapitulate the key advances in the production of SC-derived platelets using several SC sources, and discuss some strategies that apply three-dimensional bioreactor devices and biochemical factors synergistically to improve the generation of large-scale platelets for use in future biomedical and clinical settings.

Key words: Megakaryopoiesis; Platelet production; Transfusion; Bioreactors; Stem cells

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Core tip: Platelets derived from voluntary blood donation pose some challenges, such as susceptibility to pathogen contamination, short preservation time and difficulty in satisfying the increasing number of patients requiring platelet transfusion. Thus, seeking a safe and reliable alternative source of platelets is an effective solution.

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INTRODUCTION

Platelets are the smallest anucleate cells (2-4 μm in diameter) produced by megakaryocytes (MKs) in bone marrow (BM). They play a pivotal role not only in hemostasis and thrombosis but also in neoangiogenesis, innate immunity and inflammation. Until now, platelet transfusion, which may lead to varying degrees of hemorrhage that is sometimes life-threatening, has remained the most effective way to treat patients suffering from thrombocytopenia and/or platelet dysfunction^[1,2]. However, platelets derived from voluntary blood donation pose some challenges, including susceptibility to pathogen contamination, short preservation time, and difficulty satisfying the increasing number of patients requiring platelet transfusion^[3]. Therefore, seeking a safe and reliable alternative source of platelets is an effective solution.

In recent years, stem cells (SCs) research has developed rapidly in the field of regenerative medicine, and several research groups have focused on cultured platelet production *ex vivo*. In addition to hematopoietic SCs (HSCs), human embryonic SCs (hESCs) and human induced pluripotent SCs (iPSCs) have been considered potential sources for generating human MKs^[4-6] and platelets^[7,8]. The proper use of biochemical stimuli, including growth factors and cytokines, could augment MK maturation and platelet generation during *ex vivo* stem cell culturing^[9,10]. To mimic the physical microenvironment, some bioengineering techniques that promote MK maturation and platelet release have been developed^[11,12]. In this review, we will recapitulate the methods that promise to produce sufficient platelets differentiated from SCs *ex vivo* and some obstacles in this field. We believe that the *ex vivo* production of platelets is a state-of-the-art technology integrated with the development of unlimited SCs, newly discovered biochemical reagents and three-dimensional (3D) bioreactor systems.

MEGAKARYOPOIESIS AND PLATELET FORMATION

Megakaryopoiesis and thrombopoiesis are elaborate processes that can be divided into several successive stages: The commitment of SCs toward the MK lineage, proliferation of MK progenitors, MK maturation, proplatelet formation and terminal platelet production. *In vivo*, HSCs located in the osteoblastic niche on the surface of the bone cavity have the ability for self-renewal and multipotent differentiation. They give rise to common lymphoid progenitors and common myeloid progenitors, which differentiate into granulocyte/macrophage progenitors and MKs-erythrocyte progenitors (MEPs). MEPs subsequently commit to the erythrocytic and megakaryocytic lineages, which progressively produce different ages of immature MKs with various proliferative potency. The unique feature of megakaryocytic maturation is endomitosis due to a failure in cytokinesis (not all MKs undergo mitosis). This process is accompanied by an increase in DNA content (up to 128 N) and the following events: Cell enlargement; the emergence of various unique organelles, such as dense bodies and secretory particles; the synthesis and assembly of cytoplasmic proteins; the formation of the demarcation membrane system; and the formation of a membrane reservoir for proplatelets. Upon maturation, MKs migrate to the vascular niche and extend cytoplasmic projections (proplatelets) into the sinusoidal endothelium after cytoskeletal remodeling. Under shear forces produced by the blood stream, proplatelets are released, gradually develop into mature platelets and undergo reassembly and the displacement of microtubules^[13-15] (Figure 1).

In vitro, SCs (HSCs, hESCs or iPSCs) are cultured to increase progenitor cell number before differentiation into MKs. After expansion, SCs differentiate into the megakaryocytic lineage as a result of stimulation with growth factors [*e.g.*, thrombopoietin (THPO) and stem cell factor (SCF)] and MK progenitor expansion with a recombinant human THPO mimetic (AMG531)^[16]. Immature MKs then undergo the polyploidization process by inducing endomitosis to increase the number of DNA content to 16-128 N. The myosin inhibitor nicotinamide, actin inhibitors and Src inhibitor could be used for MK polyploidization. However, the capacity of MK to undergo polyploidization in culture is significantly lower than in the BM micro-

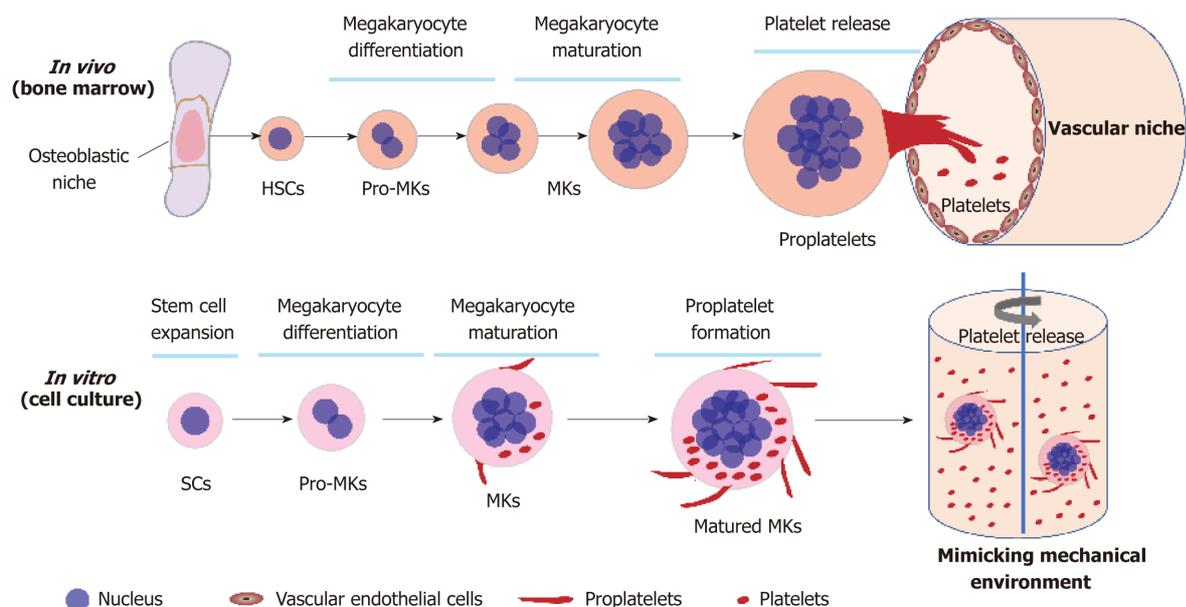


Figure 1 Overview of megakaryopoiesis, proplatelet formation and platelet release. *In vivo*, bone marrow is located within the trabecular bones, where the hematopoietic stem cell (HSC) undergo the process of megakaryocyte (MK) differentiation and MK maturation under the control of thrombopoietin. During maturation, MKs migrate to bone marrow sinusoids and form proplatelets. Proplatelets elongate through the vascular endothelium into the vessels, and proplatelet terminal ends are released into the bloodstream by blood shear forces, forming platelets. *In vitro*, HSC-derived CD34⁺ cells or pluripotent stem cell are first expanded *in vitro* and then differentiate into the megakaryocytic lineage. Once differentiated, immature MKs undergo the polyploidization process *via* inducing endomitosis to increase the number of DNA content to 16-128 N. During the process of polyploidization, some extended proplatelets are formed and released in high ploidy MKs in culture. To increase the efficiency of platelets, MKs extend proplatelets into the bioreactor, with perfused culture medium mimicking blood flow.

environment, with a maximum ploidy of 126 N in hESC-derived MKs and 16 N in iPSC-derived cells^[17,18]. During the process of polyploidization, some proplatelets are formed and released in high ploidy MKs in culture. There is a connection between apoptosis and the process of proplatelet formation because the intrinsic apoptosis pathway is necessary for MKs to adequately acquire proplatelets and release platelets^[19,20]. To promote the maturation of MKs and accelerate the release of platelets, scientists have altered the culture conditions, including by treating the cells with chemical substances and mimicking the physical microenvironment (Figure 1).

STEM CELL SOURCES FOR PLATELET PRODUCTION EX VIVO

Pluripotent SCs and hematopoietic progenitors are the main sources that have been used to generate MKs and functional platelets. The former, including hESCs and hiPSCs, is considered an unlimited seeding cell source. The latter involves CD34⁺ (a surface marker usually expressed by hematopoietic stem/progenitor cells) cells from BM, umbilical cord blood (UCB) and peripheral blood (PB). Because they are primary cells, there is a risk of viral contamination. In total, each of these sources has advantages and disadvantages for clinical platelet transfusion.

HSCs

CD34⁺ cells extracted from BM, PB and UCB are simple and feasible sources for platelet production *ex vivo*. In 1995, Choi *et al*^[21] isolated CD34⁺ cells from human PB and cocultured them with serum taken from dogs suffering from aplastic anemia. Upon the generation of MKs, human AB serum (AB-HS) was added to the culture system to promote the production of platelet-like particles. When adenosine diphosphate (ADP) was added, aggregation could be observed. This is the first report of human functional platelet production *in vitro*. Since then, the application of THPO has accelerated the development of research on platelet generation *ex vivo*. The scientists found that the addition of different cytokines in the culture medium, except for thrombopoietin, could improve megakaryocytic maturation and platelet production. To some extent, the ultrastructure and physiological functions of derived platelets were similar to those of platelets *in vivo*. However, because of the inconvenience of obtaining BM samples and because the content of CD34⁺ cells is low in the medullary cavity, the ability to induce CD34⁺ cells from BM is greatly

restricted^[22-25]. In comparison, cord blood has been widely used because of its extensive sources, convenient collection and high amplification efficiency *ex vivo*. Matsunaga *et al*^[22] reported that when CB CD34⁺ cells were cocultured with hTERT stromal cells and interleukin-11 (IL-11), SCF, thrombopoietin and flt-3 ligand (FL) were included in the system, large-scale generation of human platelets could be attained. Using the UCB differentiation system, scientists have investigated various factors that may influence megakaryopoiesis and thrombopoiesis. Employing UCBs depends on donation after childbirth, which makes it more difficult to acquire abundant materials to scale platelet production and increases the risks of bacterial/viral contamination^[26].

hESCs

Since the establishment of the hES cell line for the first time by Thomson *et al*^[27] in 1998, scientists have successively attempted to induce hESCs into MKs and platelets. In 2001, Kaufman *et al*^[28] cocultured the hES cell line H1 with S17 mouse BM stromal cells to produce CD34⁺ hematopoietic precursor cells, which can give rise to mixed cell clones containing MKs. This was the first report on the differentiation of hESCs into MKs. Then, Gaur *et al*^[4] reported that hESCs could be differentiated into MKs by culturing them with OP-9 stromal cells. After two weeks, CD41a⁺CD42b⁺ (surface marker expressed by MKs) cells were detected in the differentiation system. However, these MKs did not exceed a ploidy of 32 N, and proplatelet formation was only occasionally observed. These results suggested that the MKs were immature and that platelets were not produced in the culture system. In 2008, successful generation of platelets was addressed in a study by Takayama's group^[29]. Through coculture with OP9 or C3H10T1/2 stromal cells and the use of vascular endothelial growth factor (VEGF), hESCs produced many "sac" structures that could provide a suitable microenvironment for hematopoietic progenitor cells. Thrombopoietin, SCF and heparin were then added to the system. After approximately 10 days, platelets that could be activated by ADP or thrombin were produced in the supernatant. Therefore, the efficiency of platelet production in MKs is far less than that under physiological conditions.

In 2011, Lu *et al*^[7] improved the differentiation efficiency of MKs from hESCs by 20-30 times. The breakthrough of the experiment was that the adhesion and aggregation functions of platelets produced *ex vivo* was proven to some extent. More importantly, the cultured platelets were observed to participate in the formation of thrombosis with platelets *in vivo* at the site of damage in arterial blood vessels. However, the application of stromal cells and serum in previous studies reduced the possibility of use in therapies significantly. Subsequently, Pick *et al*^[30] committed hESCs to MKs that fragmented into platelet-like particles using a "spin embryoid body" method in serum-free differentiation medium. However, this method also has disadvantages, namely system instability and limitations to the large-scale production of MKs.

hiPSCs

Compared with hESCs, hiPSCs have several advantages, including the ability to utilize any somatic cell that could develop into MKs and the absence of ethical restrictions. In 2010, Takayama *et al*^[18] first reported that when cultured iPSCs derived from human epidermal fibroblasts with a mouse C3H10T1/2 stromal cell line for 22-26 d, platelets could be released from MKs. It is noteworthy that the platelets were observed to form thrombi at the site of damaged vessels in combination with platelets *in vivo*. In addition, hiPSCs from subcutaneous adipose tissues^[31], endometrial stromal SCs^[32] and adipose-derived mesenchymal stromal/stem cell line^[33] could differentiate into functional platelets.

In addition to the above-mentioned stromal cell coculture methods and traditional "embryoid body" differentiation methods, some strategies of altering cell fate by transcription factors have also made progress. Ono *et al*^[34] reported that overexpression of the *p45NF-E2*, *Maf G* and *Maf K* genes could convert human and mouse 3T3 fibroblasts into CD41⁺ MKs, which can produce platelet-like particles with partial coagulation function. Although the method requires much time and the efficiency is low, it showed that exogenous gene manipulation could directly transform other cell types into MKs. The key was to find the appropriate genes. In 2014, Nakamura *et al*^[8] showed that overexpression of the *BMI1*, *bcl-xl* and *c-myc* genes in hiPSCs derived from PB cells could generate stable and immortalized megakaryo-cytic progenitor cell lines (imMKCLs). Once the expression of the three genes was stopped, the imMKCLs gave rise to platelets. Similarly, ectopic expression of the three transcription factors GATA1, FLI1, and TAL1 in hiPSCs achieved the same goal, leading to mature MK production with unprecedented efficiency^[6]. Both cell lines could be cryopreserved and expanded upon recovery. Compared to MKs derived from iPSCs, the prominent feature of imMKCLs is the generation of platelets with higher efficiency in less time.

Although some characteristics of the imMKCLs deserve recommendation, the cell lines still face some drawbacks, such as relying on serum and feeder cell culture and virus-mediated genetic reprogramming, which may pose potent risks. Recently, important progress has been made by Liu *et al*^[35], who developed an efficient system to generate MKs from hiPSCs under feeder-free and xeno-free conditions by adding FDA-approved pharmacological reagents, including romiplostim (Nplate, a THPO analog), oprelvekin (recombinant IL-11), and plas-bumin (human albumin). However, platelet production from iPSCs requires relatively complex and sophisticated methods, and the culture period is long. These results may impede the progress of the industrial-scale generation of cultured platelets and therapeutic applications in regenerative medicine.

REGULATION FACTORS FOR MEGAKARYOCYTIC MATURATION AND PLATELET RELEASE

Megakaryopoiesis and thrombopoiesis are regulated by various effectors in the BM microenvironment, such as stromal cells, cytokines, extracellular matrix and blood flow. Based on theoretical knowledge, researchers have altered the culture conditions, including chemical substances, and the physical microenvironment, which may promote the differentiation and maturation of MKs and accelerate the release of platelets^[14,36,37].

Megakaryocytic maturation

It is generally recognized that the ploidy of MKs is positively correlated with the number of platelets produced. Studies have shown that MKs differentiated from CB, hESCs or hiPSCs have a far lower ploidy level than MKs generated from BM HSCs^[38,39], and the reason has not been fully elucidated. Insights into the signaling pathways associated with megakaryocytic polyploidy may contribute to our understanding and result in the production of more platelets *ex vivo* through gene manipulation^[40,41].

The Rho/ROCK pathway is a well-known regulator of the actin cytoskeleton. Chang *et al*^[42] provided evidence that the pathway acts as a negative regulator of proplatelet formation. Overexpression of a spontaneously active RhoA or dominant-negative mutation led to an increase or a decrease in proplatelet formation. The application of the Rho inhibitor Tat-C3 and the ROCK inhibitor Y27362 promoted the proportion of polyploidy MKs and the formation of proplatelets by decreasing myosin light chain 2 (MLC2) phosphorylation. Another study showed that ROCK inhibition drives polyploidization and proplatelet formation in MKs maturation through downregulation of *NFE2* and *MYC* expression^[43].

The tumor suppressor protein P53 plays an important role in regulating the cell cycle and apoptosis^[44]. P53 activation inhibits the induction of hematopoietic progenitors and of MKs^[45]. Fuhrken *et al*^[46] found that in the differentiation of the megakaryocytic cell line CHRF-288-11 induced by PMA, reducing the level of P53 improved the proportion of polyploid MKs. In addition, BM HSCs from P53^{-/-} mice can generate 64 N MKs, while the maximum ploidy of wild-type mice is 32 N. Later, Giammona *et al*^[17] showed niacinamide (NIC) can promote polyploidization of MKs by inhibition of SIRT1 and/or SIRT2, which belongs to the histone deacetylase Sirtuins protein family. The function of SIRT1 and SIRT2 is to deacetylate the downstream target protein P53. Therefore, the role of NIC in promoting polyploidization may be related to the enhanced transcriptional activity of P53 after acetylation.

Lannutti *et al*^[47] found that Fyn and Lyn, members of the Src family of protein kinases, were highly expressed during the differentiation of BM CD34⁺/CD38^{lo} cells to MKs. Lyn-deficient mice produced more mature MKs above 8 N in the presence of the Src kinase inhibitor pyrrolopyrimidine 1. In addition, the Src kinase inhibitor SU6656 increased the proportion of polyploid MKs in the differentiation of cell lines UT-7, HEL, and Meg-01 and in patients with myeloid dysplasia syndrome^[48,49].

In addition, some other important molecules, such as DIAPH1 and Gfi1b, also regulate major functions of MK proplatelet formation by controlling the dynamics of the actin and microtubule cytoskeletons^[50,51].

Platelet release

Currently, only a few MKs cultured *ex vivo* can release platelets. Therefore, it is essential to improve the ability of MKs to produce platelets *ex vivo*. Factors related to platelet release include the extracellular matrix, blood flow shear force, and MK apoptosis.

There has been evidence that the apoptotic inhibitory proteins Bcl-2 and Bcl-xl are

expressed in the early phase of megakaryopoiesis, but Bcl-2 is absent in neonatal and mature platelets. Moreover, the activity of caspase-3 and caspase-9 are increased in terminally differentiated MKs, suggesting that the maturation of MKs is accompanied by apoptosis^[52]. However, Josefsson *et al.*^[20] found that the platelet numbers in *Bak* and *Bax* knockout mice were normal. It seems that platelet production is independent of intrinsic apoptotic pathway activation. In addition, White *et al.*^[53] reported that the number and function of platelets in *Casp9*^{-/-} mice was not affected. Therefore, whether MK apoptosis affects platelet release remains to be investigated.

The extracellular matrix can modulate the production of proplatelets. Type I collagen in the osteoblast niche may inhibit the formation of proplatelets by $\alpha 5\beta 1\alpha 4\beta 1$. Fibroblast growth factor-4 (FGF-4) enhances the adhesion between MKs and vascular endothelium, contributing to the survival and maturation of MKs. By chemotaxis of stromal cell-derived factor 1 (SDF1), MKs migrate to the vascular niche and release platelets. In addition, von Willebrand factor (vWF) and fibrinogen play a role in promoting the generation of proplatelets through GPIb-IX-V and $\alpha IIb\beta 3$ signaling pathways, respectively^[54-57]. On the other hand, the podosome could adhere to extracellular matrix substrates and degrade it, which might play a role in proplatelet arm extension or penetration of the basement membrane^[58].

Mechanic stress is an important factor in platelet release from mature MKs^{59,60}. Junt *et al.*^[61] observed the process of MKs interplaying with blood vessels to produce platelets in real time by multiphoton intravital microscopy. In addition, confocal and electron microscopy after fixation were used. They found that MK exposure to high shear rates promotes platelet production *via* GPIb, which depends on microtubule elongation and assembly. The results provided a theoretical foundation for the application of bioreactors. Dunois *et al.*^[62] suggested that high shear rates from blood flow promote platelet production *via* GPIb, which depends on microtubule assembly and elongation. Recently, Ito *et al.*^[60] reported that turbulence activates platelet biogenesis and that turbulent flow promotes platelet release from hiPSC-derived MKs, suggesting turbulence as an important physical regulator in thrombopoiesis.

Platelet function

For cultured platelets to be considered for clinical application, they must be very close to donor-derived platelets in terms of quantity and quality. To date, cultured platelets *ex vivo* have fragmented function compared with donors, although ultrastructure and surface markers are similar. The testing of platelet function has mostly relied on the measurement of P-selectin exposure and GPIIb-IIIa activation. In general, a large proportion of cultured platelets are expression of activation markers by agonists stimulating such as ADP or thrombin, while we often observed that a state of pre-activation with P-selection expression in cultured platelets even if it's in the absence of any agonist and thus show poor transfusion properties^[63,64]. In addition, platelets generated *ex vivo* can participate in the formation of thrombosis in the site of damage in blood vessels in *NOG* mice. However, the degree to which these platelets can play an active role in hemostasis remains to be evaluated.

Ideally, platelets cultured *ex vivo* should be evaluated using a standard platelet aggregation assay that requires a platelet count of at least $100 \times 10^9/L$, which has not been reported by the research group. Furthermore, the loss of platelet membrane surface glycoproteins such as GPIb is another important factor that interferes with platelet function. The matrix protein metalloprotease inhibitor GM6001 can prevent GPIIb protein hydrolysis by ADAMTS17 and improve platelet function^[65,66]. Based on the current situation, platelets collected *ex vivo* have at least some physiological functions, but avoiding self-activation in culture is a difficult challenge.

PROGRESS AND FUTURE DIRECTIONS IN PLATELET BIOREACTORS

Compared with platelets in PB, the platelets produced *in vitro* were more heterogeneous in size and were produced at a lower output. One explanation is the static quality of cell culture, which is unlike the microenvironment *in vivo*. A reasonable approach for large-scale platelet production is to combine bioreactors with chemical factors. Different bioreactors recapitulate different physiological conditions (Table 1), including gas exchange, media perfusion^[67], extracellular matrix proteins, scaffold composition and the effects of blood shear stress^[68-71].

In 2003, Li *et al.*^[72] first discovered that a murine CCE ES cell line could be used to drive hematopoietic cells in a 3D fibrous matrix to direct hematopoietic differentiation using specific cytokines and inhibitors. In 2009, Sullenbarger *et al.*^[23] reported that a 3D bioreactor with surgical-grade woven polyester fabric or purpose-built hydrogel

Table 1 Some major advances in bioreactors in platelet formation and platelet release from 2013 to 2018

Type of bioreactor	Principles and methods	Designers / users
Two-directional flow bioreactor	The bioreactor consisted of two-directional flow, in which the angle between the directions of the main and pressure flow is 60 degrees	Nakagawa <i>et al.</i> ^[74] , 2013
Microfluidic platelet bioreactor	The bioreactor is based on polydimethylsiloxane bonded to glass slides to construct some upper and lower microfluidic channel	Thon <i>et al.</i> ^[70] , 2014
Spinning-membrane filtration device	Separating <i>in-vitro</i> -derived PLTs and recovering immature MKs and the precursor cells of PLTs by use of spinning-membrane filtration device	Schlinker <i>et al.</i> ^[77] , 2015
RCCS bioreactor	Shear force, simulated microgravity, and better diffusion of nutrients and oxygen from the RCCS	Yang <i>et al.</i> ^[67] , 2016
Innovative bioreactor	The bioreactor consisted of a membrane, and using flow through the membrane and shear across the membrane to drive the megakaryocytes to release PLTs	Avanzi <i>et al.</i> ^[43] , 2014
Microfluidic device	This device consists in a wide array of von Willebrand factor-coated micropillars, allowing them to remain trapped and subjected to hydrodynamic shear	Blin <i>et al.</i> ^[75] , 2016
Turbulent flow-based bioreactor	Stimulation with optimized shear stress and turbulent energy, collaborates with several growth factors for proplatelet formation	Ito <i>et al.</i> ^[60] , 2018

RCCS: Rotary cell culture system.

scaffolds could facilitate platelet output when coated with THPO with/or fibronectin. Subsequently, Lasky *et al.*^[73] designed the bioreactor by optimizing oxygen concentrations and media perfusion to promote platelet output, but they did not consider blood shear stress. Later, Nakagawa *et al.*^[74] developed a two-directional flow bioreactor and found that two flows in different directions could promote platelet production by as much as 3.6-fold compared with static cultures. In 2014, Thon *et al.*^[70] made a microfluidic platelet bioreactor that attempted to use biomimetic BM and blood vessel microenvironments and supported live imaging for platelet generation. Blin *et al.*^[75] built on previous work, reporting a bioreactor consisting of a wide array of vWF-coated micropillars to act as anchors on MKs. MKs were anchored and subjected to shear stress. Elongation of MK cytoplasm and proplatelet formation were observed. However, these devices have mostly focused on the development of proof-of-concept basic research, low throughput, custom-made tools.

Based on the concept that human MKs are partly regulated by the extracellular matrix, scientists began to design bioreactors with different materials to emulate BM physiology. To increase platelet production, Pallotta *et al.*^[57,76] applied biocompatible silk microtubes with fibrinogen, type 1 collagen, and SDF1, mimicking the release of platelets in a blood vessel. The MKs extended proplatelets through the micropores of the microtube and released platelets when exposed to low shear stress. Additionally, silk sponges^[64], polycarbonate filter membranes^[77] or the hyaluronan-based hydrogels^[78] were also used to mimic the bioengineered 3D BM environment, while there are major limitations to implementing the material in biomedical devices.

The combination of bioreactors and chemical factors might promote megakaryo/thrombopoiesis. Recently, we used the rotary cell culture system (RCCS), a unique 3D cell culture method, to investigate the potential role in megakaryopoiesis. Our results indicated that RCCS significantly improves the efficiency of platelet generation, which recapitulates some special characteristics, including shear force, simulated microgravity, and better diffusion of nutrients and oxygen. Additionally, we demonstrated that RCCS combined with chemical compounds and growth factors identified *via* small screening can further increase platelet generation efficiency^[68], while there is limitation to lacking the function of automatic change of medium. Analogously, a novel bioreactor with a membrane and three ports of input and output was developed and can produce a high number of platelets from UCB-derived CD34⁺ cells. Based on *in vivo* imaging, Ito *et al.*^[60] found that turbulence is an important physical regulator of thrombopoiesis. They developed a turbulent flow-based bioreactor (VerMES Bioreactor), which enabled high yield and quality *ex vivo* biogenesis from imMKCLs. In addition, three novel chemical factors (IGFBP2, MIF, and NRDC) that contribute to the remodeling of mature MKs and shedding of

platelets might represent a potential mechanism to promote proplatelet shedding in the VerMES bioreactor^[61]. Thus, next-generation platelet bioreactor need to be designed with comprehensive consideration fluid dynamics modeling, automation of flow control and avoid the too highly cost of device design and cell culture.

CONCLUSION

As the demand for platelet transfusions increases, many countries are competing to improve the efficiency of platelet production *ex vivo*. However, the field of megakaryopoiesis and platelet research still faces several limitations, including (1) The massive number of platelets (100-300 billion platelets) needed for one transfusion; (2) the high economic cost for the entire culture period; and (3) the lower viability of *ex vivo* platelets compared to donor-derived platelets, though they express the proper surface marker for platelet function and participate in the aggregation reaction at the site of damage in a mouse model. Optimizing the key steps of megakaryopoiesis and platelet generation may provide a better understanding of the cellular and molecular mechanisms. Introducing SCs into advanced bioreactors and simultaneous exposure to a subset of chemical compounds may synergistically contribute to the production of a large number of platelets for clinical applications. In addition, before clinical application, the platelet function produced *ex vivo* must be defined in detail and fully verified. Collection, cost-effective and highly controllable strategies and methodologies represent an important step toward large-scale platelet production for future biomedical and clinical applications.

REFERENCES

- 1 Stasi R. How to approach thrombocytopenia. *Hematology Am Soc Hematol Educ Program* 2012; **2012**: 191-197 [PMID: 23233580 DOI: 10.1182/asheducation-2012.1.191]
- 2 Goel R, Chopra S, Tobian AAR, Ness PM, Frank SM, Cushing M, Vasovic L, Kaicker S, Takemoto C, Josephson CD, Nellis M, Bussel J, Krishnamurti L. Platelet transfusion practices in immune thrombocytopenia related hospitalizations. *Transfusion* 2019; **59**: 169-176 [PMID: 30520045 DOI: 10.1111/trf.15069]
- 3 Lambert MP, Sullivan SK, Fuentes R, French DL, Poncz M. Challenges and promises for the development of donor-independent platelet transfusions. *Blood* 2013; **121**: 3319-3324 [PMID: 23321255 DOI: 10.1182/blood-2012-09-455428]
- 4 Gaur M, Kamata T, Wang S, Moran B, Shattil SJ, Leavitt AD. Megakaryocytes derived from human embryonic stem cells: a genetically tractable system to study megakaryocytopoiesis and integrin function. *J Thromb Haemost* 2006; **4**: 436-442 [PMID: 16420577 DOI: 10.1111/j.1538-7836.2006.01744.x]
- 5 Smith BW, Murphy GJ. Stem cells, megakaryocytes, and platelets. *Curr Opin Hematol* 2014; **21**: 430-437 [PMID: 25023469 DOI: 10.1097/MOH.0000000000000064]
- 6 Moreau T, Evans AL, Vasquez L, Tijssen MR, Yan Y, Trotter MW, Howard D, Colzani M, Arumugam M, Wu WH, Dalby A, Lampela R, Bouet G, Hobbs CM, Pask DC, Payne H, Ponomaryov T, Brill A, Soranzo N, Ouweland WH, Pedersen RA, Ghevaert C. Large-scale production of megakaryocytes from human pluripotent stem cells by chemically defined forward programming. *Nat Commun* 2016; **7**: 11208 [PMID: 27052461 DOI: 10.1038/ncomms11208]
- 7 Lu SJ, Li F, Yin H, Feng Q, Kimbrel EA, Hahm E, Thon JN, Wang W, Italiano JE, Cho J, Lanza R. Platelets generated from human embryonic stem cells are functional in vitro and in the microcirculation of living mice. *Cell Res* 2011; **21**: 530-545 [PMID: 21221130 DOI: 10.1038/cr.2011.8]
- 8 Nakamura S, Takayama N, Hirata S, Seo H, Endo H, Ochi K, Fujita K, Koike T, Harimoto K, Dohda T, Watanabe A, Okita K, Takahashi N, Sawaguchi A, Yamanaka S, Nakauchi H, Nishimura S, Eto K. Expandable megakaryocyte cell lines enable clinically applicable generation of platelets from human induced pluripotent stem cells. *Cell Stem Cell* 2014; **14**: 535-548 [PMID: 24529595 DOI: 10.1016/j.stem.2014.01.011]
- 9 Avezilla ST, Hattori K, Heissig B, Tejada R, Liao F, Shido K, Jin DK, Dias S, Zhang F, Hartman TE, Hackett NR, Crystal RG, Witte L, Hicklin DJ, Bohlen P, Eaton D, Lyden D, de Sauvage F, Rafii S. Chemokine-mediated interaction of hematopoietic progenitors with the bone marrow vascular niche is required for thrombopoiesis. *Nat Med* 2004; **10**: 64-71 [PMID: 14702636 DOI: 10.1038/nm973]
- 10 Kaushansky K. Lineage-specific hematopoietic growth factors. *N Engl J Med* 2006; **354**: 2034-2045 [PMID: 16687716 DOI: 10.1056/NEJMra052706]
- 11 Malara A, Balduini A. Blood platelet production and morphology. *Thromb Res* 2012; **129**: 241-244 [PMID: 22226434 DOI: 10.1016/j.thromres.2011.11.042]
- 12 Flaumenhaft R. Bioengineering in platelet biology. *Thromb Res* 2014; **133**: 523-524 [PMID: 24433610 DOI: 10.1016/j.thromres.2013.12.036]
- 13 Kosaki G. Platelet production by megakaryocytes: proplatelet theory justifies cytoplasmic fragmentation model. *Int J Hematol* 2008; **88**: 255-267 [PMID: 18751873 DOI: 10.1007/s12185-008-0147-7]
- 14 Geddis AE. Megakaryopoiesis. *Semin Hematol* 2010; **47**: 212-219 [PMID: 20620431 DOI: 10.1053/j.seminhematol.2010.03.001]
- 15 Deutsch VR, Tomer A. Advances in megakaryocytopoiesis and thrombopoiesis: from bench to bedside. *Br J Haematol* 2013; **161**: 778-793 [PMID: 23594368 DOI: 10.1111/bjh.12328]
- 16 Currao M, Balduini CL, Balduini A. High doses of romiplostim induce proliferation and reduce proplatelet formation by human megakaryocytes. *PLoS One* 2013; **8**: e54723 [PMID: 23359807 DOI: 10.1371/journal.pone.0054723]
- 17 Giammona LM, Panuganti S, Kemper JM, Apostolidis PA, Lindsey S, Papoutsakis ET, Miller WM.

- Mechanistic studies on the effects of nicotinamide on megakaryocytic polyploidization and the roles of NAD⁺ levels and SIRT inhibition. *Exp Hematol* 2009; **37**: 1340-1352.e3 [PMID: 19715739 DOI: 10.1016/j.exphem.2009.08.004]
- 18 **Takayama N**, Nishimura S, Nakamura S, Shimizu T, Ohnishi R, Endo H, Yamaguchi T, Otsu M, Nishimura K, Nakanishi M, Sawaguchi A, Nagai R, Takahashi K, Yamanaka S, Nakauchi H, Eto K. Transient activation of c-MYC expression is critical for efficient platelet generation from human induced pluripotent stem cells. *J Exp Med* 2010; **207**: 2817-2830 [PMID: 21098095 DOI: 10.1084/jem.20100844]
- 19 **White MJ**, Kile BT. Apoptotic processes in megakaryocytes and platelets. *Semin Hematol* 2010; **47**: 227-234 [PMID: 20620433 DOI: 10.1053/j.seminhematol.2010.03.006]
- 20 **Josefsson EC**, James C, Henley KJ, Debrincat MA, Rogers KL, Dowling MR, White MJ, Kruse EA, Lane RM, Ellis S, Nurden P, Mason KD, O'Reilly LA, Roberts AW, Metcalf D, Huang DC, Kile BT. Megakaryocytes possess a functional intrinsic apoptosis pathway that must be restrained to survive and produce platelets. *J Exp Med* 2011; **208**: 2017-2031 [PMID: 21911424 DOI: 10.1084/jem.20110750]
- 21 **Choi ES**, Nichol JL, Hokom MM, Hornkohl AC, Hunt P. Platelets generated in vitro from proplatelet-displaying human megakaryocytes are functional. *Blood* 1995; **85**: 402-413 [PMID: 7529062]
- 22 **Matsunaga T**, Tanaka I, Kobune M, Kawano Y, Tanaka M, Kuribayashi K, Iyama S, Sato T, Sato Y, Takimoto R, Takayama T, Kato J, Ninomiya T, Hamada H, Niitsu Y. Ex vivo large-scale generation of human platelets from cord blood CD34⁺ cells. *Stem Cells* 2006; **24**: 2877-2887 [PMID: 16960134 DOI: 10.1634/stemcells.2006-0309]
- 23 **Sullenbarger B**, Bahng JH, Gruner R, Kotov N, Lasky LC. Prolonged continuous in vitro human platelet production using three-dimensional scaffolds. *Exp Hematol* 2009; **37**: 101-110 [PMID: 19013002 DOI: 10.1016/j.exphem.2008.09.009]
- 24 **Norol F**, Vitrat N, Cramer E, Guichard J, Burstein SA, Vainchenker W, Debili N. Effects of cytokines on platelet production from blood and marrow CD34⁺ cells. *Blood* 1998; **91**: 830-843 [PMID: 9446643]
- 25 **van den Oudenrijn S**, von dem Borne AE, de Haas M. Differences in megakaryocyte expansion potential between CD34(+) stem cells derived from cord blood, peripheral blood, and bone marrow from adults and children. *Exp Hematol* 2000; **28**: 1054-1061 [PMID: 11008018 DOI: 10.1016/S0301-472X(00)00517-8]
- 26 **Thon JN**, Medvetz DA, Karlsson SM, Italiano JE. Road blocks in making platelets for transfusion. *J Thromb Haemost* 2015; **13** Suppl 1: S55-S62 [PMID: 26149051 DOI: 10.1111/jth.12942]
- 27 **Thomson JA**, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; **282**: 1145-1147 [PMID: 9804556 DOI: 10.1126/science.282.5391.1145]
- 28 **Kaufman DS**, Hanson ET, Lewis RL, Auerbach R, Thomson JA. Hematopoietic colony-forming cells derived from human embryonic stem cells. *Proc Natl Acad Sci USA* 2001; **98**: 10716-10721 [PMID: 11535826 DOI: 10.1073/pnas.191362598]
- 29 **Takayama N**, Nishikii H, Usui J, Tsukui H, Sawaguchi A, Hiroyama T, Eto K, Nakauchi H. Generation of functional platelets from human embryonic stem cells in vitro via ES-sacs, VEGF-promoted structures that concentrate hematopoietic progenitors. *Blood* 2008; **111**: 5298-5306 [PMID: 18388179 DOI: 10.1182/blood-2007-10-117622]
- 30 **Pick M**, Azzola L, Osborne E, Stanley EG, Elefanty AG. Generation of megakaryocytic progenitors from human embryonic stem cells in a feeder- and serum-free medium. *PLoS One* 2013; **8**: e55530 [PMID: 23424635 DOI: 10.1371/journal.pone.0055530]
- 31 **Matsubara Y**, Murata M, Ikeda Y. Culture of megakaryocytes and platelets from subcutaneous adipose tissue and a preadipocyte cell line. *Methods Mol Biol* 2012; **788**: 249-258 [PMID: 22130712 DOI: 10.1007/978-1-61779-307-317]
- 32 **Wang J**, Chen S, Zhang C, Stegeman S, Pfaff-Amesse T, Zhang Y, Zhang W, Amesse L, Chen Y. Human endometrial stromal stem cells differentiate into megakaryocytes with the ability to produce functional platelets. *PLoS One* 2012; **7**: e44300 [PMID: 22952951 DOI: 10.1371/journal.pone.0044300]
- 33 **Tozawa K**, Ono-Uruga Y, Yazawa M, Mori T, Murata M, Okamoto S, Ikeda Y, Matsubara Y. Megakaryocytes and platelets from a novel human adipose tissue-derived mesenchymal stem cell line. *Blood* 2019; **133**: 633-643 [PMID: 30487128 DOI: 10.1182/blood-2018-04-842641]
- 34 **Ono Y**, Wang Y, Suzuki H, Okamoto S, Ikeda Y, Murata M, Poncz M, Matsubara Y. Induction of functional platelets from mouse and human fibroblasts by p45NF-E2/Maf. *Blood* 2012; **120**: 3812-3821 [PMID: 22855609 DOI: 10.1182/blood-2012-02-413617]
- 35 **Liu Y**, Wang Y, Gao Y, Forbes JA, Qayyum R, Becker L, Cheng L, Wang ZZ. Efficient generation of megakaryocytes from human induced pluripotent stem cells using food and drug administration-approved pharmacological reagents. *Stem Cells Transl Med* 2015; **4**: 309-319 [PMID: 25713465 DOI: 10.5966/sctm.2014-0183]
- 36 **Kaushansky K**. Thrombopoiesis. *Semin Hematol* 2015; **52**: 4-11 [PMID: 25578413 DOI: 10.1053/j.seminhematol.2014.10.003]
- 37 **Huang N**, Lou M, Liu H, Avila C, Ma Y. Identification of a potent small molecule capable of regulating polyploidization, megakaryocyte maturation, and platelet production. *J Hematol Oncol* 2016; **9**: 136 [PMID: 27927231 DOI: 10.1186/s13045-016-0358-y]
- 38 **Mattia G**, Vulcano F, Milazzo L, Barca A, Macioce G, Giampaolo A, Hassan HJ. Different ploidy levels of megakaryocytes generated from peripheral or cord blood CD34⁺ cells are correlated with different levels of platelet release. *Blood* 2002; **99**: 888-897 [PMID: 11806991 DOI: 10.1182/blood.V99.3.888]
- 39 **Trakala M**, Rodríguez-Acebes S, Maroto M, Symonds CE, Santamaría D, Ortega S, Barbacid M, Méndez J, Malumbres M. Functional reprogramming of polyploidization in megakaryocytes. *Dev Cell* 2015; **32**: 155-167 [PMID: 25625205 DOI: 10.1016/j.devcel.2014.12.015]
- 40 **Johnson B**, Fletcher SJ, Morgan NV. Inherited thrombocytopenia: novel insights into megakaryocyte maturation, proplatelet formation and platelet lifespan. *Platelets* 2016; **27**: 519-525 [PMID: 27025194 DOI: 10.3109/09537104.2016.1148806]
- 41 **Espasandin YR**, Glembofsky AC, Grodzielski M, Lev PR, Goette NP, Molinas FC, Marta RF, Heller PG. Anagrelide platelet-lowering effect is due to inhibition of both megakaryocyte maturation and proplatelet formation: insight into potential mechanisms. *J Thromb Haemost* 2015; **13**: 631-642 [PMID: 25604267 DOI: 10.1111/jth.12850]
- 42 **Chang Y**, Auradé F, Larbret F, Zhang Y, Le Couedic JP, Momeux L, Larghero J, Bertoglio J, Louache F, Cramer E, Vainchenker W, Debili N. Proplatelet formation is regulated by the Rho/ROCK pathway. *Blood* 2007; **109**: 4229-4236 [PMID: 17244674 DOI: 10.1182/blood-2006-04-020024]
- 43 **Avanzi MP**, Goldberg F, Davila J, Langhi D, Chiattonne C, Mitchell WB. Rho kinase inhibition drives megakaryocyte polyploidization and proplatelet formation through MYC and NFE2 downregulation. *Br J*

- Haematol* 2014; **164**: 867-876 [PMID: 24383889 DOI: 10.1111/bjh.12709]
- 44 **Toledo F**, Wahl GM. Regulating the p53 pathway: in vitro hypotheses, in vivo veritas. *Nat Rev Cancer* 2006; **6**: 909-923 [PMID: 17128209 DOI: 10.1038/nrc2012]
- 45 **Mahfoudhi E**, Lordier L, Marty C, Pan J, Roy A, Roy L, Rameau P, Abbes S, Debili N, Raslova H, Chang Y, Debussche L, Vainchenker W, Plo I. P53 activation inhibits all types of hematopoietic progenitors and all stages of megakaryopoiesis. *Oncotarget* 2016; **7**: 31980-31992 [PMID: 26959882 DOI: 10.18632/oncotarget.7881]
- 46 **Fuhrken PG**, Apostolidis PA, Lindsey S, Miller WM, Papoutsakis ET. Tumor suppressor protein p53 regulates megakaryocytic polyploidization and apoptosis. *J Biol Chem* 2008; **283**: 15589-15600 [PMID: 18397889 DOI: 10.1074/jbc.M801923200]
- 47 **Lannutti BJ**, Drachman JG. Lyn tyrosine kinase regulates thrombopoietin-induced proliferation of hematopoietic cell lines and primary megakaryocytic progenitors. *Blood* 2004; **103**: 3736-3743 [PMID: 14726379 DOI: 10.1182/blood-2003-10-3566]
- 48 **Lannutti BJ**, Blake N, Gandhi MJ, Reems JA, Drachman JG. Induction of polyploidization in leukemic cell lines and primary bone marrow by Src kinase inhibitor SU6656. *Blood* 2005; **105**: 3875-3878 [PMID: 15677565 DOI: 10.1182/blood-2004-10-3934]
- 49 **Kaminska J**, Klimczak-Jajor E, Skierski J, Bany-Laszewicz U. Effects of inhibitor of Src kinases, SU6656, on differentiation of megakaryocytic progenitors and activity of alpha1,6-fucosyltransferase. *Acta Biochim Pol* 2008; **55**: 499-506 [PMID: 18854874]
- 50 **Pan J**, Lordier L, Meyran D, Rameau P, Lecluse Y, Kitchen-Goosen S, Badirou I, Mokrani H, Narumiya S, Alberts AS, Vainchenker W, Chang Y. The formin DIAPH1 (mDia1) regulates megakaryocyte proplatelet formation by remodeling the actin and microtubule cytoskeletons. *Blood* 2014; **124**: 3967-3977 [PMID: 25298036 DOI: 10.1182/blood-2013-12-544924]
- 51 **Beauchemin H**, Shooshtarizadeh P, Vadnais C, Vassen L, Pastore YD, Möröy T. Gfi1b controls integrin signaling-dependent cytoskeleton dynamics and organization in megakaryocytes. *Haematologica* 2017; **102**: 484-497 [PMID: 28082345 DOI: 10.3324/haematol.2016.150375]
- 52 **Patel SR**, Hartwig JH, Italiano JE. The biogenesis of platelets from megakaryocyte proplatelets. *J Clin Invest* 2005; **115**: 3348-3354 [PMID: 16322779 DOI: 10.1172/JCI26891]
- 53 **White MJ**, Schoenwaelder SM, Josefsson EC, Jarman KE, Henley KJ, James C, Debrincat MA, Jackson SP, Huang DC, Kile BT. Caspase-9 mediates the apoptotic death of megakaryocytes and platelets, but is dispensable for their generation and function. *Blood* 2012; **119**: 4283-4290 [PMID: 22294729 DOI: 10.1182/blood-2011-11-394858]
- 54 **Malara A**, Gruppi C, Rebuzzini P, Visai L, Perotti C, Moratti R, Balduini C, Tira ME, Balduini A. Megakaryocyte-matrix interaction within bone marrow: new roles for fibronectin and factor XIII-A. *Blood* 2011; **117**: 2476-2483 [PMID: 21131589 DOI: 10.1182/blood-2010-06-288795]
- 55 **Mazharian A**, Wang YJ, Mori J, Bem D, Finney B, Heising S, Gissen P, White JG, Berndt MC, Gardiner EE, Nieswandt B, Douglas MR, Campbell RD, Watson SP, Senis YA. Mice lacking the ITIM-containing receptor G6b-B exhibit macrothrombocytopenia and aberrant platelet function. *Sci Signal* 2012; **5**: ra78 [PMID: 23112346 DOI: 10.1126/scisignal.2002936]
- 56 **Balduini A**, Pallotta I, Malara A, Lova P, Pecci A, Viarengo G, Balduini CL, Torti M. Adhesive receptors, extracellular proteins and myosin IIA orchestrate proplatelet formation by human megakaryocytes. *J Thromb Haemost* 2008; **6**: 1900-1907 [PMID: 18752571 DOI: 10.1111/j.1538-7836.2008.03132.x]
- 57 **Pallotta I**, Lovett M, Kaplan DL, Balduini A. Three-dimensional system for the in vitro study of megakaryocytes and functional platelet production using silk-based vascular tubes. *Tissue Eng Part C Methods* 2011; **17**: 1223-1232 [PMID: 21895494 DOI: 10.1089/ten.tec.2011.0134]
- 58 **Schachtner H**, Calaminus SD, Sinclair A, Monypenny J, Blundell MP, Leon C, Holyoake TL, Thrasher AJ, Michie AM, Vukovic M, Gachet C, Jones GE, Thomas SG, Watson SP, Machesky LM. Megakaryocytes assemble podosomes that degrade matrix and protrude through basement membrane. *Blood* 2013; **121**: 2542-2552 [PMID: 23305739 DOI: 10.1182/blood-2012-07-443457]
- 59 **Holme PA**, Orvim U, Hamers MJ, Solum NO, Brosstad FR, Barstad RM, Sakariassen KS. Shear-induced platelet activation and platelet microparticle formation at blood flow conditions as in arteries with a severe stenosis. *Arterioscler Thromb Vasc Biol* 1997; **17**: 646-653 [PMID: 9108776 DOI: 10.1161/01.ATV.17.4.646]
- 60 **Ito Y**, Nakamura S, Sugimoto N, Shigemori T, Kato Y, Ohno M, Sakuma S, Ito K, Kumon H, Hirose H, Okamoto H, Nogawa M, Iwasaki M, Kihara S, Fujio K, Matsumoto T, Higashi N, Hashimoto K, Sawaguchi A, Harimoto KI, Nakagawa M, Yamamoto T, Handa M, Watanabe N, Nishi E, Arai F, Nishimura S, Eto K. Turbulence Activates Platelet Biogenesis to Enable Clinical Scale Ex Vivo Production. *Cell* 2018; **174**: 636-648.e18 [PMID: 30017246 DOI: 10.1016/j.cell.2018.06.011]
- 61 **Junt T**, Schulze H, Chen Z, Massberg S, Goerge T, Krueger A, Wagner DD, Graf T, Italiano JE, Shivdasani RA, von Andrian UH. Dynamic visualization of thrombopoiesis within bone marrow. *Science* 2007; **317**: 1767-1770 [PMID: 17885137 DOI: 10.1126/science.1146304]
- 62 **Dunois-Lardé C**, Capron C, Fichelson S, Bauer T, Cramer-Bordé E, Baruch D. Exposure of human megakaryocytes to high shear rates accelerates platelet production. *Blood* 2009; **114**: 1875-1883 [PMID: 19525480 DOI: 10.1182/blood-2009-03-209205]
- 63 **Feng Q**, Shabrani N, Thon JN, Huo H, Thiel A, Machlus KR, Kim K, Brooks J, Li F, Luo C, Kimbrel EA, Wang J, Kim KS, Italiano J, Cho J, Lu SJ, Lanza R. Scalable generation of universal platelets from human induced pluripotent stem cells. *Stem Cell Reports* 2014; **3**: 817-831 [PMID: 25418726 DOI: 10.1016/j.stemcr.2014.09.010]
- 64 **Di Buduo CA**, Wray LS, Tozzi L, Malara A, Chen Y, Ghezzi CE, Smoot D, Sfara C, Antonelli A, Spedden E, Bruni G, Staii C, De Marco L, Magnani M, Kaplan DL, Balduini A. Programmable 3D silk bone marrow niche for platelet generation ex vivo and modeling of megakaryopoiesis pathologies. *Blood* 2015; **125**: 2254-2264 [PMID: 25575540 DOI: 10.1182/blood-2014-08-595561]
- 65 **Bergmeier W**, Burger PC, Piffath CL, Hoffmeister KM, Hartwig JH, Nieswandt B, Wagner DD. Metalloproteinase inhibitors improve the recovery and hemostatic function of in vitro-aged or -injured mouse platelets. *Blood* 2003; **102**: 4229-4235 [PMID: 12907434 DOI: 10.1182/blood-2003-04-1305]
- 66 **Nishikii H**, Eto K, Tamura N, Hattori K, Heissig B, Kanaji T, Sawaguchi A, Goto S, Ware J, Nakauchi H. Metalloproteinase regulation improves in vitro generation of efficacious platelets from mouse embryonic stem cells. *J Exp Med* 2008; **205**: 1917-1927 [PMID: 18663123 DOI: 10.1084/jem.20071482]
- 67 **Yang Y**, Liu C, Lei X, Wang H, Su P, Ru Y, Ruan X, Duan E, Feng S, Han M, Xu Y, Shi L, Jiang E, Zhou J. Integrated Biophysical and Biochemical Signals Augment Megakaryopoiesis and Thrombopoiesis in a Three-Dimensional Rotary Culture System. *Stem Cells Transl Med* 2016; **5**: 175-185 [PMID: 26702125]

- DOI: [10.5966/sctm.2015-0080](https://doi.org/10.5966/sctm.2015-0080)]
- 68 **Sugimoto N**, Eto K. Platelet production from induced pluripotent stem cells. *J Thromb Haemost* 2017; **15**: 1717-1727 [PMID: [28752663](https://pubmed.ncbi.nlm.nih.gov/28752663/) DOI: [10.1111/jth.13736](https://doi.org/10.1111/jth.13736)]
- 69 **Thon JN**, Dykstra BJ, Beaulieu LM. Platelet bioreactor: accelerated evolution of design and manufacture. *Platelets* 2017; **28**: 472-477 [PMID: [28112988](https://pubmed.ncbi.nlm.nih.gov/28112988/) DOI: [10.1080/09537104.2016.1265922](https://doi.org/10.1080/09537104.2016.1265922)]
- 70 **Thon JN**, Mazutis L, Wu S, Sylman JL, Ehrlicher A, Machlus KR, Feng Q, Lu S, Lanza R, Neeves KB, Weitz DA, Italiano JE. Platelet bioreactor-on-a-chip. *Blood* 2014; **124**: 1857-1867 [PMID: [25606631](https://pubmed.ncbi.nlm.nih.gov/25606631/) DOI: [10.1182/blood-2014-05-574913](https://doi.org/10.1182/blood-2014-05-574913)]
- 71 **Wong AK**. Platelet biology: the role of shear. *Expert Rev Hematol* 2013; **6**: 205-212 [PMID: [23547868](https://pubmed.ncbi.nlm.nih.gov/23547868/) DOI: [10.1586/ehm.13.5](https://doi.org/10.1586/ehm.13.5)]
- 72 **Li Y**, Ma T, Kniss DA, Yang ST, Lasky LC. Human cord cell hematopoiesis in three-dimensional nonwoven fibrous matrices: in vitro simulation of the marrow microenvironment. *J Hematother Stem Cell Res* 2001; **10**: 355-368 [PMID: [11454311](https://pubmed.ncbi.nlm.nih.gov/11454311/) DOI: [10.1089/152581601750288966](https://doi.org/10.1089/152581601750288966)]
- 73 **Lasky LC**, Sullenbarger B. Manipulation of oxygenation and flow-induced shear stress can increase the in vitro yield of platelets from cord blood. *Tissue Eng Part C Methods* 2011; **17**: 1081-1088 [PMID: [21877917](https://pubmed.ncbi.nlm.nih.gov/21877917/) DOI: [10.1089/ten.tec.2011.0108](https://doi.org/10.1089/ten.tec.2011.0108)]
- 74 **Nakagawa Y**, Nakamura S, Nakajima M, Endo H, Dohda T, Takayama N, Nakauchi H, Arai F, Fukuda T, Eto K. Two differential flows in a bioreactor promoted platelet generation from human pluripotent stem cell-derived megakaryocytes. *Exp Hematol* 2013; **41**: 742-748 [PMID: [23618622](https://pubmed.ncbi.nlm.nih.gov/23618622/) DOI: [10.1016/j.exphem.2013.04.007](https://doi.org/10.1016/j.exphem.2013.04.007)]
- 75 **Blin A**, Le Goff A, Magniez A, Poirault-Chassac S, Teste B, Sicot G, Nguyen KA, Hamdi FS, Reyssat M, Baruch D. Microfluidic model of the platelet-generating organ: beyond bone marrow biomimetics. *Sci Rep* 2016; **6**: 21700 [PMID: [26898346](https://pubmed.ncbi.nlm.nih.gov/26898346/) DOI: [10.1038/srep21700](https://doi.org/10.1038/srep21700)]
- 76 **Pallotta I**, Kluge JA, Moreau J, Calabrese R, Kaplan DL, Balduini A. Characteristics of platelet gels combined with silk. *Biomaterials* 2014; **35**: 3678-3687 [PMID: [24480538](https://pubmed.ncbi.nlm.nih.gov/24480538/) DOI: [10.1016/j.biomaterials.2013.12.065](https://doi.org/10.1016/j.biomaterials.2013.12.065)]
- 77 **Schlinker AC**, Radwanski K, Wegener C, Min K, Miller WM. Separation of in-vitro-derived megakaryocytes and platelets using spinning-membrane filtration. *Biotechnol Bioeng* 2015; **112**: 788-800 [PMID: [25312394](https://pubmed.ncbi.nlm.nih.gov/25312394/) DOI: [10.1002/bit.25477](https://doi.org/10.1002/bit.25477)]
- 78 **Currao M**, Malara A, Di Buduo CA, Abbonante V, Tozzi L, Balduini A. Hyaluronan based hydrogels provide an improved model to study megakaryocyte-matrix interactions. *Exp Cell Res* 2016; **346**: 1-8 [PMID: [26027944](https://pubmed.ncbi.nlm.nih.gov/26027944/) DOI: [10.1016/j.yexcr.2015.05.014](https://doi.org/10.1016/j.yexcr.2015.05.014)]



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