Transfusion and Apheresis Science xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

In vitro platelet production for transfusion purposes: Where are we now?

Patricia Martínez-Botía[a,b], Andrea Acebes-Huerta[a], Jerard Seghatchian[c,1], Laura Gutiérrez[a,b,*,1]

[a] Platelet Research Lab, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain
[b] Dept. of Medicine, University of Oviedo, Spain
[c] International Consultancy in Strategic Advices on Safety Improvements of Blood-Derived Bioproducts and Suppliers Quality Audit / Inspection, London, England, UK

ARTICLE INFO

Keywords:
Platelet transfusion
Megakaryocyte differentiation
In vitro PLT culture
Primary culture development
Cell reprogramming

ABSTRACT

Over the last decade there has been a worldwide increase in the demand of platelet concentrates (PCs) for transfusion. This is, to a great extent, due to a growing and aging population with the concomitant increase in the incidence of onco-hematological diseases, which require frequent platelet (PLT) transfusions. Currently, PLTs are sourced uniquely from donations, and their storage time is limited only to a few days. The necessity to store PCs at room temperature (to minimize loss of PLT functional integrity), poses a major risk for bacterial contamination. While the implementation of pathogen reduction treatments (PRTs) and new-generation PLT additive solutions have allowed the extension of the shelf life and a safer PLT transfusion product, the concern of PCs shortage still pressures the scientific community to find alternative solutions with the aim of meeting the PLT transfusion increasing demand. In this concise report, we will focus on the efforts made to produce, in *in vitro* culture, high yields of viable and functional PLTs for transfusion purposes in a cost-effective manner, meeting not only current Good Manufacturing Practices (cGMPs), but also transfusion safety standards.

1. The culture nature and cellular sources

1.1. Neither homogenous nor synchronous culture

Platelets (PLTs) derive from bone marrow megakaryocytes (MKs), which undergo a complex differentiation process driven mainly by the hormone thrombopoietin (TPO) [1]. Interestingly, the TPO receptor, MPL, is not restricted to the MK committed precursors, but it is also expressed in earlier hematopoietic precursors and stem cells (HSCs) [2], regulating their self-renewal, proliferation and differentiation [3,4]. It is common to observe that when aiming at the *in vitro* differentiation of MKs from hematopoietic precursors, cultures are highly heterogeneous (*i.e.* they are enriched in MKs at different stages of differentiation but still containing other hematopoietic cells).

The fact that there is still residual megakaryopoiesis in Tpo-deficient mice, suggests the existence of alternative TPO-independent mechanisms driving megakaryopoiesis, which have been extensively studied using genetic strategies in mouse models [5,6]. As a consequence, a number of published MK culture methods consider the addition of several growth factors along with a variety of cytokines, including granulocyte-macrophage colony stimulating factor (GM-CSF), interleukins, erythropoietin (EPO), stem cell factor (SCF) and/or Flt3-ligand (FLT3-L) [7,8], which seem to cooperate with TPO in the process of megakaryocyte proliferation, differentiation, maturation and PLT release in different ways. However, as recently reported and observed by us (unpublished data), megakaryopoiesis under subjacent inflammation differs from megakaryopoiesis in healthy conditions, and ultimately results in PLTs displaying a distinct functional profile [9]. It is in this situation that IL-1α and other inflammatory cytokines modulate megakaryopoiesis [10]. Therefore, it is important to acknowledge that adding some of these cytokines to the culture would create an inflammatory environment that will directly affect megakaryopoiesis and its PLT produce [11]. Unfortunately, at the present time, there is still not a consensus on which factors are needed in order to efficiently mimic physiological PLT production *in vitro*, to provide steady state competent PLTs at the functional level, through more enriched and synchronous cultures. A thorough characterization at the molecular and functional level of the MKs and PLTs obtained with the different growth factor cocktails would be the basis to reach such a consensus.

Additionally, the harvesting of the produced PLTs all at once, pure, free of debris and vesicles, and the maintenance of their functional integrity is a recognizable bottleneck. Current methods developed to tackle this issue contemplate serial centrifugations at different time points [12]. This technique, however, might result in yield reduction,

* Corresponding author at: Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Edificio FINBA, Platelet Research Lab NO-F15, Avenida de Roma S/N, 33011, Oviedo, Spain.
E-mail address: gutierrezglaura@uniovi.es (L. Gutiérrez).
[1] Equal contribution

https://doi.org/10.1016/j.transci.2020.102864

PLT activation, and it is time-consuming and difficult to standardize to meet cGMP requirements. Recently, a procedure involving a spinning-membrane filtration device was developed that ensures the return of a high yield of pure and functional PLTs [13], and more advances are expected to be reported in the near future in this respect.

1.2. Species and developmental stage

To date, most of the knowledge generated to understand the mechanisms governing megakaryopoiesis derives from studies using mouse models, and at this moment we sit at the tip of the iceberg regarding the comprehensive characterization of megakaryopoiesis in human [14,15]. It is of the utmost importance to acknowledge that, while many processes find their parallelism in an inter-species manner, there are always species-specific peculiarities, and we should be able to understand the human models when studying a disease or designing a therapy.

Most of the limitations on this front, in addition to the heterogeneous and asynchronous nature of MK cultures, are the intrinsic and distinct characteristics of MKs (and PLTs) differentiated *in vitro* from different tissues, especially considering the developmental stage [fetal liver, umbilical cord blood (CB), peripheral blood (PB) and bone marrow (BM)] [16]. Of note, it is easier to grow and differentiate MKs from fetal tissues, compared to those derived from adult tissues. However, MKs of fetal origin are smaller, and although mature in granule content, they reach lower ploidy levels, as compared to MKs of adult origin [17]. Interestingly, fetal/neonatal and adult PLTs have shown distinct transcriptomes and functional response profiles [18–20].

When thinking of the appropriate cell source to generate PLTs for transfusion in *in vitro* culture, it is important to bear in mind the major developmental, phenotypical and transcriptional differences in megakaryopoiesis between cells from fetal/neonatal and adult origin, which will undoubtedly render PLTs with different phenotypes and functional characteristics, with the consequent impact on the final transfusion product [21].

1.3. Primary or reprogrammed hematopoietic precursors

The *ex vivo* production of PLTs focused first on the differentiation of primary human $CD34^+$ hematopoietic stem cells (HSCs), which have been successfully isolated from umbilical cord blood (CB), adult peripheral blood (PB) and from bone marrow (BM) [22–24]. CB $CD34^+$ cells, although limited due to their availability and authorized access, have been the most widely used source for human MK culture *in vitro*. Subsequent developments and modifications in the growth conditions allowed for the culture of MKs from both PB and BM (*i.e.* adult tissues) [25]. While the relative frequency of $CD34^+$ cells in PB is lower than in CB samples [26], it can be enriched by G-CSF-induced mobilization of $CD34^+$ progenitors from the BM [12]. In addition, other whole blood processed materials may provide with a more enriched $CD34^+$ cell fraction, such as leuko-depletion filters and buffy coats from routine donations [27,28]. BM $CD34^+$ cells require invasive techniques for harvesting and seem unable to sustain a large yield of MKs in culture, although they might represent the most appropriate source for recapitulating physiological MK differentiation from a given patient *ex vivo* [29,30]. For many years, $CD34^+$ cell sorting was a standard prerequisite for hematopoietic cell culture, but it is important to bear in mind that $CD34^+$ sorted cells do not constitute a pure HSC population, however enriched. In particular, a $CD34^+$ enriched cell fraction does not assure a homogeneous nor synchronous MK culture either. Not surprisingly, MKs can differentiate from the crude fraction of human peripheral blood mononuclear cells (PMBCs), which contain hematopoietic progenitors, reducing the costs of the process [8].

However, primary cells are not immortalized, and cultures get exhausted, which is *per se* a limitation when the ultimate goal is to produce high numbers of PLTs [31]. The next logical step on the quest of optimizing *in vitro* PLT production occurred with the incorporation of cell reprogramming to the picture, among them human pluripotent stem cells (hPSCs), which comprise human embryonic stem cells (hESCs) [32] and the recently discovered human induced pluripotent stem cells (iPSCs) [33]. Both can be differentiated towards any cell type, given adequate growth conditions, including MKs and PLT-like particles [34,35]. As a drawback, the embryonic character of the cell culture presents a risk of malignant transformation or teratoma formation [36], and while the collected PLTs can be irradiated to prevent remaining cells from causing any damage, a large number of apoptotic cells and vesicles in the culture supernatant may elicit an immune response when transfused [37]. Of note, a full comprehensive characterization of MKs and PLTs derived from these reprogrammed sources (as compared to steady state adult or neonatal PLTs) is lacking.

Currently, ethical concerns, sophisticated experimental techniques, and costs pose a barrier in the justification of this cell source for PLT production with transfusion purposes [32,38]. Their growth requirements for serum and feeder cells make them unsuitable to be produced abiding cGMP guidelines. While still not in a cost-effective manner, advances have been made to adapt the culture methods to feeder- and serum-free conditions, as some studies have shown [39]. Still, they present with advantages that outweigh their drawbacks, such as being an inexhaustible, self-renewable source of MKs and PLTs [38]. They can also be genetically modified to, for example, match any major histocompatibility group or, contrariwise, to be devoid of HLA antigen to give rise to universal PLTs, minimizing the risk of refractoriness and alloimmunization [40].

1.4. Non-hematopoietic sources

Fibroblasts, endothelial and adipose tissue-derived stromal cells (ASCs) have been used as source material to generate *in vitro* MKs with various culture techniques [41–43]. Of the three, ASCs seem to perform better. ASCs differentiate into MKs in a cost-effective way by means of endogenous TPO, and without genetic manipulation or feeder cells [43]. Resulting PLTs were found to be functional, with normal PLT surface marker expression and the PLT output was comparable to that obtained with iPSCs. The major drawbacks present in this study were that only a subpopulation of ASCs was able to differentiate into MKs, and that they were non self-renewable. A second generation cell line (ASCL) has been generated by the same group that overcomes some of the issues identified [44].

2. The culture system and engineering

Another aspect of concern regarding *in vitro* PLT production is the physical conditions of the culture system. Currently, PLT yields obtained *in vitro* are far from those encountered in physiological conditions and it is mainly due to an inefficient release from *in vitro* cultured MKs, which adds to the lack of synchronicity and controllable MK terminal differentiation in culture (when considering that a MK may release in the order of 1000 PLTs). Ongoing efforts focus on overcoming this bottleneck by reproducing the complex interactions that occur within the BM through bioengineering [45].

Bioreactors represent state-of-the-art technology aimed at recapitulating megakaryopoiesis and at manufacturing PLTs in large quantities [46]. In the last years, the main effort to mimic the BM environment has focused on developing microfluidic bioreactors to faithfully reflect its key physiological characteristics, such as the extracellular matrix (ECM) composition, BM stiffness, soluble factors and blood vessel architecture, which includes tissue-specific microvascular endothelium, endothelial cell contacts and circulatory shear stress [47]. They constitute the most suitable method for large-scale production due to its scalability, handling, and the possibility to control the cell density, the nutrient distribution and the culture conditions (*i.e.* pH, temperature, and O_2 and CO_2 concentrations) [48,49]. When switching from 2D

to 3D cultures there is an increment of the surface area, which in turn, allows for more interactions between MKs and their proplatelets, and the surrounding co-cultured cells. To achieve this kind of environment, several scaffolds have been used, made of hydrogel [50], polyester, or polydimethylsiloxane (PDMS) [51], coated with ECM proteins (*e.g.* fibronectin, collagen, von Willebrand factor), and perfused with media containing specific growth factors and cytokines [47].

The diversity of synthetic or biological microfluidic devices and co-culture systems shows the audacity and ingenious developments of the scientific community [52,53]. Reports show that optimal MK differentiation and production is directly influenced by several variables (*e.g.* hypoxia, hypothermia, shear stress or turbulence) and that an accurate control of the physical attributes of the bioreactor is crucial [48,54–56]. However, as of today, there is not a consensus on which system or physical conditions might be the most appropriate for the purpose.

3. Alternatives to the alternative

3.1. Using the lungs as in vivo bioreactors

An alternative and already tested approach, is turning the lungs into *in vivo* bioreactors by intravenously infusing *ex vivo* cultured MKs, and using the pulmonary bed as the location of PLT production [57–59]. From studies performed in mice, MKs were found entrapped in the lung vasculature, and a small percentage was found in the spleen, while there were no signs of entrapment in either the BM, liver, heart, or brain. PLTs released under these circumstances were comparable to donor-derived PLTs in terms of size, granule distribution, half-life, and surface marker expression; however, they were released in a delayed manner, were rapidly cleared from the circulation and appeared hyporeactive to certain stimuli [59].

The approach of allowing the differentiation and late stages of PLT production in the host upon MK infusion is promising, and phase I Clinical Trials have studied their safety and tolerability with positive results [60]. This therapeutic option would allow the manipulation of MKs in culture (*i.e.* genetic/molecular modification), prior infusion, to provide the host with a specific set of PLTs. However, the approach as of today poses its own risks. The number of mature MKs required to achieve sensitive PLT corrected count increment (CCI), could potentially cause capillary obstruction and their extruded nucleus may elicit inflammation and autoimmune responses [59]. Another concern would be the time it takes for these infused MKs to produce PLTs (approximately 24 h), a delay that might not always be clinically affordable in comparison to the immediate effect of a PLT transfusion.

3.2. Artificial platelets

The development of artificial PLTs, seems a promising alternative for the acute bleeding patients requiring immediate transfusions, as this hybrid synthetic PLTs are compatible with all blood groups, comply with transfusion safety requirements, are biodegradable and their production costs are more reduced than those of *in vitro* PLT production (considering a single application unit) [61]. Another objective that directs the scientific community to future developments in bio-engineering, is the Holy Grail of tailoring ¨smart¨ PLTs with specific cargo or functional features, so that they could target specific tissues or cells, and contribute to physiological processes related to and beyond hemostasis [62].

4. Concluding remarks

In recent years there have been enormous advances towards the manufacturing of PLTs *ex vivo*, in order to meet the rising demand of PLT transfusions. Despite these advances, there are still hurdles that must be overcome. Costs and lengthy production (up to 26 days), added to the fact that their shelf life is even shorter than their donated counterparts, makes them unsuitable in cases of emergency, where large numbers of PLTs are needed, in a very short period of time. Furthermore, a more thorough characterization of both MKs and PLTs produced *in vitro* is required. Many studies have focused on the PLT yield, while obviating either their morphology, immunophenotype or functionality, both *in vitro* and *in vivo*. Furthermore, these *ex vivo* PLTs have yet to be fully tested in humans. Clinical trials have only focused so far on their safety and tolerability, meaning further trials designed to assess their efficacy, function, and other specific variables must be performed.

Considering the natural PLT distinct functional profiles due to the developmental stage (infant *vs* adult) or health status of an individual (healthy or with subjacent inflammation), it opens the question as whether *in vitro* produced PLTs from which source, method and conditions should be suitable for a given specific patient and clinical circumstances. Little is known on how PLT-entities produced at different developmental stage or health status contribute to the plethora of non-hemostatic functions assigned in recent years to PLTs, or how would they function upon transfusion considering the host´s developmental stage or health status.

Furthermore, we lack knowledge on whether *in vitro* produced PLTs may be prone to acquire PLT storage lesion in the same way as donor PLTs, whether they can be stored in the same conditions and time length, and most importantly, how do we set production protocols to not only abide cGMP regulations, but also transfusion safety? Would these *in vitro* produced PLTs require PRTs, and how would that affect their integrity?

Only when taking into consideration the discussed issues and limitations, there will be relevant advancements in the field of *in vitro* PLT production for transfusion purposes. Paving the way to a better characterization at the molecular and cell biology level, and to the standardization of the methodology of cultures, will position ourselves closer to the efficient production of cGMP-grade PLTs and their final implementation in the clinical practice.

Funding

This work is partially supported by a Ramón y Cajal Fellowship (RYC-2013 – 12587, Ministerio de Economía y Competitividad – Spain) to LG, an I + D Excellence Research Project (SAF2017-85489-P, Ministerio de Ciencia, Innovación y Universidades – Spain – and Fondos FEDER) to LG and PMB, a Severo Ochoa Grant (PA-20-PF-BP19-014) to PMB and a postdoctoral ISPA 2018 intramural fellowship to AAH.

Declaration of Competing Interest

The authors declare no conflict of interest.

References

[1] Kaushansky K. Historical review: megakaryopoiesis and thrombopoiesis. Blood 2008;111:981–6.
[2] Ninos JM, Jefferies LC, Cogle CR, Kerr WG. The thrombopoietin receptor, c-Mpl, is a selective surface marker for human hematopoietic stem cells. J Transl Med 2006;4:9.
[3] Yoshihara H, Arai F, Hosokawa K, Hagiwara T, Takubo K, Nakamura Y, et al. Thrombopoietin/MPL signaling regulates hematopoietic stem cell quiescence and interaction with the osteoblastic niche. Cell Stem Cell 2007;1:685–97.
[4] Nakamura-Ishizu A, Suda T. Multifaceted roles of thrombopoietin in hematopoietic stem cell regulation. Ann N Y Acad Sci 2020;1466(1):51–8.
[5] Ng AP, Kauppi M, Metcalf D, Hyland CD, Josefsson EC, Lebois M, et al. Mpl expression on megakaryocytes and platelets is dispensable for thrombopoiesis but essential to prevent myeloproliferation. Proc Natl Acad Sci U S A. 2014;111:5884–9.
[6] Gainsford T, Nandurkar H, Metcalf D, Robb L, Begley CG, Alexander WS. The residual megakaryocyte and platelet production in c-mpl-deficient mice is not dependent on the actions of interleukin-6, interleukin-11, or leukemia inhibitory factor. Blood 2000;95:528–34.
[7] Reems JA, Pineault N, Sun S. In vitro megakaryocyte production and platelet biogenesis: state of the art. Transfus Med Rev 2010;24:33–43.

[8] Salunkhe V, Papadopoulos P, Gutiérrez L. Culture of megakaryocytes from human peripheral blood mononuclear cells. Bio-protocol 2015;5:e1639.
[9] Müller-Newen G, Stope MB, Kraus T, Ziegler P. Development of platelets during steady state and inflammation. J Leukoc Biol 2017;101:1109–17.
[10] Nishimura S, Nagasaki M, Kunishima S, Sawaguchi A, Sakata A, Sakaguchi H, et al. IL-1alpha induces thrombopoiesis through megakaryocyte rupture in response to acute platelet needs. J Cell Biol 2015;209:453–66.
[11] Couldwell G, Machlus KR. Modulation of megakaryopoiesis and platelet production during inflammation. Thromb Res 2019;179:114–20.
[12] Panuganti S, Schlinker AC, Lindholm PF, Papoutsakis ET, Miller WM. Three-stage ex vivo expansion of high-ploidy megakaryocytic cells: toward large-scale platelet production. Tissue Eng Part A 2013;19:998–1014.
[13] 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.
[14] Strassel C, Eckly A, Léon C, Moog S, Cazenave JP, Gachet C, et al. Hirudin and heparin enable efficient megakaryocyte differentiation of mouse bone marrow progenitors. Exp Cell Res 2012;318:25–32.
[15] Italiano Jr. JE, Lecine P, Shivdasani RA, Hartwig JH. Blood platelets are assembled principally at the ends of proplatelet processes produced by differentiated megakaryocytes. J Cell Biol 1999;147:1299–312.
[16] Avanzi MP, Mitchell WB. Ex vivo production of platelets from stem cells. Br J Haematol 2014;165:237–47.
[17] Bluteau O, Langlois T, Rivera-Munoz P, Favale F, Rameau P, Meurice G, et al. Developmental changes in human megakaryopoiesis. J Thromb Haemost 2013;11:1730–41.
[18] Margraf A, Nussbaum C, Sperandio M. Ontogeny of platelet function. Blood Adv 2019;3:692–703.
[19] De Cuyper IM, Meinders M, van de Vijver E, de Korte D, Porcelijn L, de Haas M, et al. A novel flow cytometry-based platelet aggregation assay. Blood 2013;121:e70–80.
[20] Caparros-Perez E, Teruel-Montoya R, Lopez-Andreo MJ, Llanos MC, Rivera J, Palma-Barqueros V, et al. Comprehensive comparison of neonate and adult human platelet transcriptomes. PLoS One 2017;12:e0183042.
[21] Davenport P, Liu ZJ, Sola-Visner M. Changes in megakaryopoiesis over ontogeny and their implications in health and disease. Platelets 2020:1–8.
[22] Guerriero R, Testa U, Gabbianelli M, Mattia G, Montesoro E, Macioce G, et al. Unilineage megakaryocytic proliferation and differentiation of purified hemato-poietic progenitors in serum-free liquid culture. Blood 1995;86:3725–36.
[23] Tao H, Gaudry L, Rice A, Chong B. Cord blood is better than bone marrow for generating megakaryocytic progenitor cells. Exp Hematol 1999;27:293–301.
[24] De Bruyn C, Delforge A, Martiat P, Bron D. Ex vivo expansion of megakaryocyte progenitor cells: cord blood versus mobilized peripheral blood. Stem Cells Dev 2005;14:415–24.
[25] De Bruyn C, Delforge A, Lagneaux L, Bron D. Characterization of CD34+ subsets derived from bone marrow, umbilical cord blood and mobilized peripheral blood after stem cell factor and interleukin 3 stimulation. Bone Marrow Transplant 2000;25:377–83.
[26] Herbein G, Sovalat H, Wunder E, Baerenzung M, Bachorz J, Lewandowski H, et al. Isolation and identification of two CD34+ cell subpopulations from normal human peripheral blood. Stem Cells 1994;12:187–97.
[27] Ivanovic Z, Duchez P, Morgan DA, Hermitte F, Lafarge X, Chevaleyre J, et al. Whole-blood leuko-depletion filters as a source of CD 34+ progenitors potentially usable in cell therapy. Transfusion 2006;46:118–25.
[28] Six KR, Sicot G, Devloo R, Feys HB, Baruch D, Compernolle V. A comparison of haematopoietic stem cells from umbilical cord blood and peripheral blood for platelet production in a microfluidic device. Vox Sang 2019;114:330–9.
[29] van den Oudenrijn S, von dem Borne AE, de Haas M. Differences in megakaryocyte expansion potential between CD34(+) stem cells derived from cord blood, per-ipheral blood, and bone marrow from adults and children. Exp Hematol 2000;28:1054–61.
[30] Miyazaki R, Ogata H, Iguchi T, Sogo S, Kushida T, Ito T, et al. Comparative analyses of megakaryocytes derived from cord blood and bone marrow. Br J Haematol 2000;108:602–9.
[31] Lambert MP, Sullivan SK, Fuentes R, French DL, Poncz M. Challenges and promises for the development of donor-independent platelet transfusions. Blood 2013;121:3319–24.
[32] Gaur M, Kamata T, Wang S, Moran B, Shattil SJ, Leavitt AD. Megakaryocytes de-rived from human embryonic stem cells: a genetically tractable system to study megakaryocytopoiesis and integrin function. J Thromb Haemost 2006;4:436–42.
[33] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–72.
[34] Hansen M, von Lindern M, van den Akker E, Varga E. Human-induced pluripotent stem cell-derived blood products: state of the art and future directions. FEBS Lett 2019;593:3288–303.
[35] Takayama N, Eto K. Pluripotent stem cells reveal the developmental biology of human megakaryocytes and provide a source of platelets for clinical application. Cell Mol Life Sci 2012;69:3419–28.
[36] Lee AS, Tang C, Rao MS, Weissman IL, Wu JC. Tumorigenicity as a clinical hurdle for pluripotent stem cell therapies. Nat Med 2013;19:998–1004.
[37] Peng Y, Martin DA, Kenkel J, Zhang K, Ogden CA, Elkon KB. Innate and adaptive immune response to apoptotic cells. J Autoimmun 2007;29:303–9.
[38] Nakamura S, Takayama N, Hirata S, Seo H, Endo H, Ochi K, et al. Expandable megakaryocyte cell lines enable clinically applicable generation of platelets from human induced pluripotent stem cells. Cell Stem Cell 2014;14:535–48.
[39] Pick M, Azzola L, Osborne E, Stanley EG, Elefanty AG. Generation of mega-karyocytic progenitors from human embryonic stem cells in a feeder- and serum-free medium. PLoS One 2013;8:e55530.
[40] Figueiredo C, Goudeva L, Horn PA, Eiz-Vesper B, Blasczyk R, Seltsam A. Generation of HLA-deficient platelets from hematopoietic progenitor cells. Transfusion 2010;50:1690–701.
[41] Lis R, Karrasch CC, Poulos MG, Kunar B, Redmond D, Duran JGB, et al. Conversion of adult endothelium to immunocompetent haematopoietic stem cells. Nature 2017;545:439–45.
[42] Ono Y, Wang Y, Suzuki H, Okamoto S, Ikeda Y, Murata M, et al. Induction of functional platelets from mouse and human fibroblasts by p45NF-E2/Maf. Blood 2012;120:3812–21.
[43] Ono-Uruga Y, Tozawa K, Horiuchi T, Murata M, Okamoto S, Ikeda Y, et al. Human adipose tissue-derived stromal cells can differentiate into megakaryocytes and platelets by secreting endogenous thrombopoietin. J Thromb Haemost 2016;14:1285–97.
[44] Tozawa K, Ono-Uruga Y, Yazawa M, Mori T, Murata M, Okamoto S, et al. Megakaryocytes and platelets from a novel human adipose tissue-derived me-senchymal stem cell line. Blood 2019;133:633–43.
[45] Pinho S, Frenette PS. Haematopoietic stem cell activity and interactions with the niche. Nat Rev Mol Cell Biol 2019;20:303–20.
[46] Ingavle G, Shabrani N, Vaidya A, Kale V. Mimicking megakaryopoiesis in vitro using biomaterials: recent advances and future opportunities. Acta Biomater 2019;96:99–110.
[47] Thon JN, Dykstra BJ, Beaulieu LM. Platelet bioreactor: accelerated evolution of design and manufacture. Platelets 2017;28:472–7.
[48] 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;17:1081–8.
[49] Kropp C, Kempf H, Halloin C, Robles-Diaz D, Franke A, Scheper T, et al. Impact of feeding strategies on the scalable expansion of human pluripotent stem cells in single-use stirred tank bioreactors. Stem Cells Transl Med 2016;5:1289–301.
[50] Sullenbarger B, Bahng JH, Gruner R, Kotov N, Lasky LC. Prolonged continuous in vitro human platelet production using three-dimensional scaffolds. Exp Hematol 2009;37:101–10.
[51] Nakagawa Y, Nakamura S, Nakajima M, Endo H, Dohda T, Takayama N, et al. Two differential flows in a bioreactor promoted platelet generation from human plur-ipotent stem cell-derived megakaryocytes. Exp Hematol 2013;41:742–8.
[52] Di Buduo CA, Wray LS, Tozzi L, Malara A, Chen Y, Ghezzi CE, et al. Programmable 3D silk bone marrow niche for platelet generation ex vivo and modeling of mega-karyopoiesis pathologies. Blood 2015;125:2254–64.
[53] Tozzi L, Laurent PA, Di Buduo CA, Mu X, Massaro A, Bretherton R, et al. Multi-channel silk sponge mimicking bone marrow vascular niche for platelet production. Biomaterials 2018;178:122–33.
[54] Pineault N, Boucher JF, Cayer MP, Palmqvist L, Boyer L, Lemieux R, et al. Characterization of the effects and potential mechanisms leading to increased megakaryocytic differentiation under mild hyperthermia. Stem Cells Dev 2008;17:483–93.
[55] Dunois-Lardé C, Capron C, Fichelson S, Bauer T, Cramer-Bordé E, Baruch D. Exposure of human megakaryocytes to high shear rates accelerates platelet pro-duction. Blood 2009;114:1875–83.
[56] Ito Y, Nakamura S, Sugimoto N, Shigemori T, Kato Y, Ohno M, et al. Turbulence activates platelet biogenesis to enable clinical scale ex vivo production. Cell 2018;174:636–48. e18.
[57] Lefrançais E, Ortiz-Muñoz G, Caudrillier A, Mallavia B, Liu F, Sayah DM, et al. The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. Nature 2017;544:105–9.
[58] Fuentes R, Wang Y, Hirsch J, Wang C, Rauova L, Worthen GS, et al. Infusion of mature megakaryocytes into mice yields functional platelets. J Clin Invest 2010;120:3917–22.
[59] Wang Y, Hayes V, Jarocha D, Sim X, Harper DC, Fuentes R, et al. Comparative analysis of human ex vivo-generated platelets vs megakaryocyte-generated platelets in mice: a cautionary tale. Blood 2015;125:3627–36.
[60] Xi J, Zhu H, Liu D, Nan X, Zheng W, Liu K, et al. Infusion of megakaryocytic pro-genitor products generated from cord blood hematopoietic stem/progenitor cells: results of the phase 1 study. PLoS One 2013;8:e54941.
[61] Hickman DA, Pawlowski CL, Shevitz A, Luc NF, Kim A, Girish A, et al. Intravenous synthetic platelet (SynthoPlate) nanoconstructs reduce bleeding and improve' golden hour' survival in a porcine model of traumatic arterial hemorrhage. Sci Rep 2018;8:3118.
[62] Wu YW, Goubran H, Seghatchian J, Burnouf T. Smart blood cell and microvesicle-based Trojan horse drug delivery: merging expertise in blood transfusion and bio-medical engineering in the field of nanomedicine. Transfus Apher Sci 2016;54:309–18.