Journal of Visualized Experiments

In vitro and in vivo methods to explore megakaryopoiesis

--Manuscript Draft--

Article Type:	Invited Methods Collection - Editorial
Manuscript Number:	JoVE63286
Full Title:	In vitro and in vivo methods to explore megakaryopoiesis
Corresponding Author:	Anita Eckly Universite de Strasbourg 1: Universite de Strasbourg Strasbourg, 67000 FRANCE
Corresponding Author's Institution:	Universite de Strasbourg 1: Universite de Strasbourg
Corresponding Author E-Mail:	Anita.Michel@efs.sante.fr
First Author:	Anita Eckly
Author Comments:	This is a short editorial article summarizing the methods published in the collection "In vitro and in vivo methods to explore megakaryopoiesis" I had problems with the next step - Current Author List. Anita ECKLY is the corresponding author
Additional Information:	
Question	**Response**
Please confirm that you have read and agree to the terms and conditions of the author license agreement that applies below:	I agree to the Author License Agreement
Please confirm that you have read and agree to the terms and conditions of the video release that applies below:	I agree to the Video Release
Abstract:	Platelets are small, anucleate blood cells, essential for hemostasis, which derive from megakaryocytes (MKs) in the bone marrow (BM). During their differentiation from hematopoietic stem cells, MKs develop into giant cells in a complex process of differentiation and maturation that includes polyploidization and cytoplasmic maturation. In the final stage of development when MKs are in contact with endothelial cells, they achieve their mature stage and extend long cytoplasmic extensions, called proplatelets, into blood vessels, which then fragment to give rise to platelets in the bloodstream. The mechanisms that regulate MK maturation and transendothelial proplatelet formation are still incompletely understood. The goal of this collection is to bring together complementary methods that will contribute to better understand the biology of MKs in vitro and in vivo .

TITLE:

In Vitro and *In Vivo* Methods to Explore Megakaryopoiesis

AUTHORS AND AFFILIATIONS:

François Lanza, Christian Gachet, Anita Eckly

[1]Université de Strasbourg, INSERM, EFS Grand Est, BPPS UMR-S 1255, FMTS, Strasbourg, France.

Email Addresses of Co-Authors:

François Lanza (francois.lanza@efs.sante.fr)
Christian Gachet (christian.gachet@efs.sante.fr)
Anita Eckly (anita.michel@efs.sante.fr)

CORRESPONDING AUTHOR:

Anita Eckly (anita.michel@efs.sante.fr)

ARTICLES DISCUSSED:

1. Pongerard, A., Mallo, L., Gachet, C., Lanza, F., Strassel, C. Leukodepletion filters-derived CD34+ cells as a cell source to study megakaryocyte differentiation and platelet formation. *Journal of Visualized Experiments: JoVE*. (171), e62499 (2021).
2. Kimmerlin, Q., Tavian, M., Gachet, C., Lanza, F., Brouard, N. Isolation of mouse megakaryocyte progenitors. *Journal of Visualized Experiments: JoVE*. (171), e62498 (2021).
3. Boscher, J., Gachet, C., Lanza, F., Léon, C. Megakaryocyte culture in 3D methylcellulose-based hydrogel to improve cell maturation and study the impact of stiffness and confinement. *Journal of Visualized Experiments: JoVE*. (171), e62511 (2021).
4. Scandola, C., Lanza, F., Gachet, C., Eckly, A. *In situ* exploration of murine megakaryopoiesis using transmission electron microscopy. *Journal of Visualized Experiments: JoVE*. (171) e62494 (2021).
5. Guinard, I., Gachet, C., Lanza, F., Léon, C., Eckly, A. Proplatelet formation dynamics of mouse fresh bone marrow explants. *Journal of Visualized Experiments: JoVE*. (171), e62501 (2021).
6. Bornert, A., Pertuy, F., Gachet, C., Lanza, F., Léon, C. In vivo two-photon imaging of megakaryocytes and proplatelets in the mouse skull bone marrow. *Journal of Visualized Experiments: JoVE*. (171), e62515 (2021)

EDITORIAL TEXT:

Platelets are small, anucleate blood cells, essential for hemostasis, which derive from megakaryocytes (MKs) in the bone marrow (BM). During their differentiation from hematopoietic stem cells, MKs develop into giant cells in a complex process of differentiation and maturation that includes polyploidization and cytoplasmic maturation. In the final stage of development when MKs are in contact with endothelial cells, they achieve their mature stage and extend long cytoplasmic extensions, called proplatelets, into blood vessels, which then fragment to give rise to platelets in the bloodstream[1,2]. The mechanisms that regulate MK maturation and transendothelial proplatelet formation are still incompletely understood. The goal of this

collection is to bring together complementary methods that will contribute to better understand the biology of MKs *in vitro* and *in vivo*.

In vitro expansion and differentiation of MKs is widely used to study the mechanisms underlying platelet biogenesis. In the protocol by Pongerard et al., the authors describe a standardized culture protocol for the differentiation of MKs from human $CD34^+$ hematopoietic progenitors. They especially emphasize that leukocyte filters available at blood centers are an exceptional source for $CD34^+$ cells collection. Their article contains several essential guidelines on how to extract $CD34^+$ cells, how to assess their purity and the importance of their seeding density. The authors also propose a calibrated pipetting method, five times in succession, to efficiently release functional platelets. Interesting applications of this method are the possibility to evaluate pharmacological compounds or to genetically manipulate $CD34^+$ cells using a CRISPR-Cas9 genome editing method[3,4]. For mouse studies, Kimmerlin et al. present a cell sorting strategy to purify MK progenitors in sufficient numbers compatible with molecular and cellular assays. The authors discuss the merits of using the iliac crest as a source of bone marrow containing a great number of hematopoietic progenitors and of performing a two-step magnetic depletion of unwanted cells prior to cell sorting using a combination of cell surface markers. A major advantage of this method is the reduction in the number of animals required to obtain highly purified MK progenitors which, for example, may be of interest to researchers studying hematopoietic progenitors' hierarchy and their differentiation. In this context, Boscher et al. describe a method to grow progenitors in a 3D medium composed of 2% methylcellulose to better mimic the *in vivo* environment. In fact, these authors have previously demonstrated that MKs grown in a 3D hydrogel achieve a greater maturation state and a greater ability to form progenitors compared to those grown in a liquid medium[5]. Critical steps are highlighted in this protocol such as the appropriate volume of methylcellulose to obtain optimal medium stiffness, the number of cells that should be equivalent in each experimental condition, and the risk of contamination. For functional verification of the maturation level, the authors explain how to resuspend the gel-cultured cells in liquid medium on the third day of culture and how to fix the cells for future analysis by immunostaining, flow cytometry or electron microscopy. Transmission electron microscopy (TEM) is the imaging approach of choice to provide high resolution ultrastructure of MKs. Here, Scandola et al. share a protocol to analyze the ultrastructure of MKs in their natural BM environment, and to quantify their cell density and score the different maturation stages. The authors use TEM to analyze the cellular events that occur when MKs interact with the sinusoidal wall, such as their short invasive podosome-like protrusions penetrating the endothelium[6]. A limitation of the method is that the initial phase of MK engagement cannot be analyzed because of the lack of specific ultrastructural features of these immature cells. As noted, this imaging approach has the advantage of using small BM samples allowing to perform multiple studies with a single mouse. As an example, the other bone marrow samples can be used for the bone marrow explant technique which is presented in this collection by Guinard et al. to study the dynamic extension of proplatelets from MKs. The strength of this method lies in the use of MKs that have developed in the BM, thus avoiding any potential misinterpretation resulting from artifacts of cell differentiation in culture. Indeed, this has been previously documented in mice with MK-restricted *MYH9* inactivation, where divergent results were found *in vitro* and *ex vivo*[7]. The authors also highlight the simple, reproducible, and rapid nature of this approach. An interesting note of their

protocol is the ability to use microscopes equipped with navigation software to facilitate tracking/quantification of MKs extending proplatelets. A complementary method of the explant model is the two-photon imaging of MKs in the mouse skull. The uniqueness of this technique is the ability to study *in vivo* the dynamic behavior of fluorescently labeled MKs in the bone marrow and to visualize proplatelet extension. This protocol has the advantage of being minimally invasive, with limited surgical interventions, thus avoiding inflammatory reactions that have been reported to impact proplatelet formation. As Bornert et al. note, one limitation of this method is the narrow depth that can be imaged due to the density of the bone, which also imposes the use of younger mice. This technique can be applied to genetically modified mice to study the role of genes of interest in *in vivo* platelet formation. For instance, the authors used this technique to demonstrate that the role of microtubules in the elongation of proplatelets differs between *in vitro* and *in vivo* conditions[8].

Abnormal MK development and platelet production can lead to thrombocytopenia or thrombocythemia, increasing the risk of bleeding or thrombosis, respectively. Continuing efforts to dissect the molecular mechanisms governing platelet biogenesis will increase our capacity to develop novel strategies for the treatment of thrombocytopenia and thrombocythemia. In the field of transfusion research, these efforts will lay the foundation to produce cultured platelets as a transfusional alternative in selected situations[9].

ACKNOWLEDGMENTS:

This work was supported by ARMESA (Association de Recherche et Développement en Médecine et Santé Publique), the European Union through the European Regional Development Fund (ERDF) and by Grant ANR-17-CE14-Megaplatprod to H.d.S and ANR-18-CE14-PlatForMechanics to C.L.

DISCLOSURES:

The authors have nothing to disclose.

REFERENCES:

1. Boscher, J. et al. Blood platelet formation at a glance. *Journal of Cell Science*. **133** (20), jcs244731 (2020).
2. Machlus, K. R., Italiano Jr., J. E. The incredible journey: From megakaryocyte development to platelet formation. *The Journal of Cell Biology*. **201** (6), 785–796 (2013).
3. Strassel, C. et al. Aryl hydrocarbon receptor-dependent enrichment of a megakaryocytic precursor with a high potential to produce proplatelets. *Blood*. **127** (18), 2231–2240 (2016).
4. Montenont, E. et al. CRISPR-edited megakaryocytes for rapid screening of platelet gene functions. *Blood Advances*. **5** (9), 2362–2374 (2021).
5. Aguilar, A. et al. Importance of environmental stiffness for megakaryocyte differentiation and proplatelet formation. *Blood*. **128** (16), 2022–2032 (2016).
6. Eckly, A. et al. Megakaryocytes use in vivo podosome-like structures working collectively to penetrate the endothelial barrier of bone marrow sinusoids. *Journal of Thrombosis and Haemostasis*. **18** (11), 2987–3001 (2020).
7. Eckly, A. et al. Proplatelet formation deficit and megakaryocyte death contribute to

thrombocytopenia in Myh9 knockout mice. *Journal of Thrombosis and Haemostasis*. **8** (10), 2243–2251 (2010).
8. Bornert, A. et al. Cytoskeletal-based mechanisms differently regulate in vivo and in vitro proplatelet formation. *Haematologica*. **106** (5), 1368–1380 (2021).
9. Strassel, C., Gachet, C., Lanza, F. On the way to in vitro platelet production. *Transfusion Clinique et Biologique*. **25** (3), 220–227 (2018).