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State of the art

In vitro generation of platelets: Where do we stand?

Génération des plaquettes in vitro : où en sommes-nous ?

C.A. Di Buduo [a,b], D.L. Kaplan [c], A. Balduini [a,b,c,*]

[a] *Department of molecular medicine, university of Pavia, Pavia, Italy*
[b] *Biotechnology, research laboratories, IRCCS San Matteo Foundation, Pavia, Italy*
[c] *Department of biomedical engineering, Tufts university, Medford, MA, USA*

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Abstract

Millions of platelets, specialized cells that participate in haemostatic and inflammatory functions, are transfused each year worldwide, but their supply is limited. Platelets are produced by megakaryocytes by extending proplatelets, directly into the bloodstream. Bone marrow structure and extracellular matrix composition together with soluble factors (e.g. Thrombopoietin) are key regulators of megakaryopoiesis by supporting cell differentiation and platelet release. Despite this knowledge, the scarcity of clinical cures for life threatening platelet diseases is in a large part due to limited insight into the mechanisms that control the developmental process of megakaryocytes and the mechanisms that govern the production of platelets within the bone marrow. To overcome these limitations, functional human tissue models have been developed and studied to extrapolate *ex vivo* outcomes for new insight on bone marrow functions *in vivo*. There are many challenges that these models must overcome, from faithfully mimicking the physiological composition and functions of bone marrow, to the collection of the platelets generated and validation of their viability and function for human use. The overall goal is to identify innovative instruments to study mechanisms of platelet release, diseases related to platelet production and new therapeutic targets starting from human progenitor cells.
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Keywords: Megakaryocytes; Platelets; Transfusion; Bioreactor; 3D models; Thrombocytopenia

Résumé

Des millions de plaquettes, cellules spécialisées qui participent aux fonctions hémostatiques et inflammatoires, sont transfusées chaque année dans le monde entier, mais leur offre est limitée. Les plaquettes sont produites par les mégacaryocytes en émettant les pro-plaquettes directement dans la circulation sanguine. La structure de la moelle osseuse et la composition de la matrice extracellulaire ainsi que les facteurs solubles (par exemple la thrombopoïétine) sont des régulateurs clés de la mégacaryopoïèse en favorisant la différenciation cellulaire et la libération de plaquettes. Malgré cette connaissance, la rareté des traitements cliniques à vie des maladies des plaquettes est en grande partie due à une connaissance limitée quant aux mécanismes qui contrôlent le processus de développement des mégacaryocytes et des mécanismes qui régissent la production de plaquettes dans la moelle osseuse. Pour surmonter ces limites, des modèles de tissus humains ont été développés et étudiés pour extrapoler ex vivo des résultats pour une nouvelle vision sur les fonctions de la moelle osseuse in vivo. Il y a beaucoup de défis que ces modèles doivent surmonter, notamment : imiter fidèlement la composition physiologique et les fonctions de la moelle osseuse, collecter les plaquettes générées et valider de leur viabilité et fonctions pour l'usage humain. L'objectif global est d'identifier des instruments innovants pour étudier les mécanismes de libération de plaquettes, les maladies liées à la production de plaquettes et de nouvelles cibles thérapeutiques à partir de cellules progénitrices humaines.
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Mots clés : Mégacaryocytes ; Plaquettes ; Transfusion ; Bioréacteur ; Modèles 3D ; Thrombopénie

* Corresponding author at: Department of molecular medicine, university of Pavia, via Forlanini 6, 27100, Pavia, Italy.
E-mail address: alessandra.balduini@unipv.it (A. Balduini).

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1. Introduction

Millions of platelet transfusions are conducted each year, yet the supply of this blood component is limited, thus patient access to treat disorders is problematic. There are also many diseases where platelet production or function are impaired, resulting in severe consequences and where there are limited clinical options available. To address these current limitations, new modes to generate functional platelets *in vitro* would provide a major benefit to many patients, as well as provide an approach to permit the systematic investigation of mechanisms involved in functional platelet formation. The current model of platelet biogenesis provides that maturing megakaryocytes (Mks) migrate from the osteoblastic niche to the bone marrow (BM) vessels, where they extend proplatelets in order to release nascent platelets directly into the bloodstream [1,2]. This process is finely regulated by the interaction of Mks with the different adhesive proteins of the BM microenvironment and by soluble factors (e.g. Thrombopoietin [TPO]) [3,4]. The BM microenvironment has a unique protein composition paired with a distinct range of stiffness. The chemical and physical cues provided by the extracellular matrix (ECM) components are key to the proper regulation of platelet production [5–7]. Although the BM has been proposed to be a major site of platelet production [2], Lefrançais et al. recently showed that in mice Mks circulate from the BM to the lungs, where they release platelets [8]. On this basis, it is becoming more and more important to shed light on the mechanisms that govern platelet production *in vivo* especially in humans where threatening platelet diseases are still cureless. Research on the BM has been hindered by technical difficulties in obtaining an intact organ without bone decalcification in mouse models or BM biopsies in humans. Therefore, the scientific community has intensively investigated *ex vivo* BM cell and tissue culture models to extrapolate such structures and functions into new insight on BM function *in vivo*. In addition, such a system would respond to the critical need for improved models to understand diseases and predict efficacy, safety and toxicology outcomes for new candidate therapeutics for treatment of platelet related diseases. Sometimes, animal models are poor predictors of efficacy and toxicology of human drugs because of interspecies differences. Therefore, specialized tools, integrating all physical and physiological elements characterizing the BM niche, are needed to study the mechanisms of action of these drugs and the overall impact on BM environment to allow future personalization of clinical treatments.

2. Bioreactors for platelet production

A number of studies point to the BM niche as the core of blood cell production but with many interesting complex environmental factors for consideration. Thus, future advancements in the study of megakaryopoiesis will depend on the evolution of bioengineering techniques for reproducing physiologically relevant conditions for mimicking the environment where platelets are released. The first biomaterial BM scaffold was constructed with colloidal crystals as a template for 3D polyacrylamide hydrogels [9]. Next, a biomimetic artificial blood vessel system was used that enabled real-time visualization of Mks during culture [10]. A microfluidic bioreactor was also established and consisted of transparent PDMS bonded to glass slides to ensure efficient gas exchange and to support high-resolution live-cell microscopy [11,12]. This system was connected to a syringe pump to create a differential flow across a 2D plastic surface into which microscopic holes have been made in order to allow platelet release in the fast flowing media. The impact of flow was also explored in a device consisting in a wide array of von Willebrand Factor-coated micropillars. Such pillars acted as anchors on Mks, allowing them to remain trapped in the device and subjected to hydrodynamic shear. The combined effect of anchoring and shear induced proplatelet elongation promote platelet release [13]. The search for a biomaterial that can be chemically and mechanically tailored to entrap bioactive molecules, while retaining bioavailability, has spawned our research into the use of silk fibroin from *Bombyx mori* silkworm cocoons, as scaffold for engineering a physiologically relevant human BM environment [14]. Silk fibroin is a naturally-derived, biocompatible and tunable biomaterial extensively used in cell culture studies, regenerative medicine and biomedical applications [15]. Fundamental characteristics of this protein include low thrombogenicity, non-toxicity and low-immunogenicity, thus making it a useful blood vessel substitute [16]. Moreover, silk can be enhanced through a variety of chemical modifications that affect cell attachment, growth and differentiation and is processed entirely in aqueous systems allowing the incorporation of cells and labile compounds without loss of bioactivity [17]. Our 3D human BM niche tissue model for functional platelet production showed that silk vascular tubes, embedded in a silk sponge mimicking the “spongy” marrow, support Mk growth and the release of platelets into the perfused vascular tube lumen [14]. Importantly, the silk was functionalized with characteristic ECM components [3,18], and Mks could sense these proteins and modify their behavior accordingly. Therefore, silk provides a unique and versatile system for reconstituting all BM features that support platelet production.

3. Strategies for functional platelet production

Future success of a bioreactor system implies the implementation with human cells capable to release safe and functional platelets. So far, the different bioengineering approaches described above have already tested different sources of Mks such as umbilical cord blood, peripheral blood and BM. All these are invaluable cell sources to understand the basic mechanisms of human platelet production both in physiologic and pathologic conditions taking advantage of the possibility to differentiate Mks form patients with platelet related diseases. The need for cells that could overcome the limits related to donor-derived platelets for transfusions have also prompted several groups to develop efficient culture systems from human induced pluripotent stem cell (hiPSCs). hiPSCs are potentially an inexhaustible source of Mks which can produce human platelets resembling peripheral blood once in many aspects, including ultrastructure, surface antigen expression, and function [11,19,20]. Further, hiPSCs can be used for studying either cell physiology or disease

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