Transfusion and Apheresis Science 53 (2015) 110–126

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

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

Review

Tips and tricks for flow cytometry-based analysis and counting of microparticles

Philippe Poncelet [a,*], Stéphane Robert [b], Nicolas Bailly [c], Francine Garnache-Ottou [d], Tarik Bouriche [a], Bérangère Devalet [e], Jerard H. Segatchian [f], Philippe Saas [d], François Mullier [c,1]

[a] Research & Technology Department, BioCytex, Marseille, France
[b] VRCM, UMR-S1076, Aix-Marseille Université, INSERM, Faculté de Pharmacie, Marseille, France
[c] Hematology Laboratory, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for Life Sciences (NARILIS), CHU UCL Namur, Université catholique de Louvain, Yvoir, Belgium
[d] INSERM U1098, EFS BFC, Université de Bourgogne Franche-Comté, Besançon, France
[e] Department of Hematology, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for Life Sciences (NARILIS), CHU UCL Namur, Yvoir, Belgium
[f] International Consultancy in Blood Component Quality/Safety Improvement, Audit/Inspection and DDR Strategy, London, UK

ARTICLE INFO

Keywords:
Microparticles
Flow cytometry
Transfusion
Labile blood products
Standardization

ABSTRACT

Submicron-sized extra-cellular vesicles generated by budding from the external cell membranes, microparticles (MPs) are important actors in transfusion as well as in other medical specialties. After briefly positioning their role in the characterization of labile blood products, this technically oriented chapter aims to review practical points that need to be considered when trying to use flow cytometry for the analysis, characterization and absolute counting of MP subsets. Subjects of active discussions relative to instrumentation will include the choice of the trigger parameter, possible standardization approaches requiring instrument quality-control, origin and control of non-specific background and of coincidence artifacts, choice of the type of electronic signals, optimal sheath fluid and sample speed. Questions related to reagents will cover target antigens and receptors, multi-color reagents, negative controls, enumeration of MPs and limiting artifacts due to unexpected (micro-) coagulation of plasma samples. Newly detected problems are generating innovative solutions and flow cytometry will continue to remain the technology of choice for the analysis of MPs, in the domain of transfusion as well as in many diverse specialties.

Contents

1. Introduction 111
2. Microparticles in labile blood products 111
   2.1. Potential functions of labile blood product-contained microparticles 111
3. Pre-analytical conditions 113
   3.1. Standardization of the pre-analytical steps 113
4. Flow cytometry: a technique amenable to standardized MP analysis? 114
   4.1. Standardizing FCM analysis of MPs: the triggering parameter is a key 114

* Corresponding author. BioCytex, 140 Chemin de l'Armée d'Afrique, 13010 Marseille, France. Tel.: +33 4 96 12 20 40; fax: +33 4 91 47 24 71.
E-mail address: philippe.poncelet@biocytex.fr (P. Poncelet).
[1] F. Mullier and Ph. Saas contributed equally.

http://dx.doi.org/10.1016/j.transci.2015.10.008
1473-0502/

4.1.1. Fluorescence 114
4.1.2. FSC-based standardization efforts 115
4.1.3. SSC-based standardization efforts 116
4.2. FCM analysis of microparticles: practical tips 118
4.2.1. FCM signals 118
4.2.2. Sample speed 119
4.2.3. Sheath fluid 119
4.2.4. Background noise 119
4.3. Reagents for flow cytometry of microparticles: problems and solutions 120
4.3.1. Target antigens and receptors 120
4.3.2. A critical problem; plasma micro-coagulation 121
4.3.3. Multi-color fluorescence: the main advantage of flow cytometry 121
4.3.4. Optimizing the dosage of reagents and negative controls 122
4.3.5. Absolute enumeration 122
5. Conclusion 123
Appendix: Supplementary material .....
References 123

1. Introduction

The aim of this review is to update technical conditions for the analysis and counting of microparticles (MPs) using flow cytometry (FCM) with the objective to apply this to the characterization of labile blood products and to clinical studies related to transfusion. We will first evoke briefly the different processes that may induce or limit the generation of MPs in labile blood products and the potential consequences of MPs in these products. Then, we will discuss pre-analytical and analytical conditions in more details.

2. Microparticles in labile blood products

Several publications report the presence of MPs, and sometimes microvesicles (or its abbreviation MVs), in labile blood products [1–29] (Table 1). Since the term of MVs is often used in the literature to define either ectosomes alone or both ectosomes and exosomes, it represents a large heterogeneous group more recently named extracellular vesicles (abbreviation EVs [31]). In this review, we will use the term of MPs to refer to ectosomes, the only ones that may be detectable by FCM. MPs correspond to particles with a diameter of less than 1 μm produced from cell membranes by a mechanism called vesiculation or budding [32]. These MPs are not only generated during apoptotic process or after cell activation [7,13], but also in response to shear conditions [33–35]. They may originate from the cells that are transfused (*i.e.*, red blood cells [RBC] [1,9,10,12,13,15,16,18,19,24,25,29] or platelets [3–7,17,21–23,26,28,36]) or from contaminants, such as leucocytes [20,33]. They can be produced *in vivo* after transfusion as a physiological process, or before transfusion as a consequence of the preparation process including: storage [1,4–7,10,12,13,15,19–21,28,29,36], filtration or leukoreduction [9,11,14,18,23,27] (Table 1) and as reviewed in this Theme [37]. Alternatively, they can also be present in the plasma to be transfused, since MPs can be detected in frozen fresh plasma (FFP) [2,11,14,20,27]. Indeed, they pre-exist in platelet-poor or platelet-free plasma (PPP) fractions obtained by centrifugation from whole blood samples and are also known as circulating MPs. The major circulating MPs found in healthy donors are platelet-derived MPs (PMPs) followed by RBC-derived MP (RMPs or Ery-MPs) [38]. Endothelial cell-derived MP (EMPs) can also be found at very low levels in blood, but their levels may increase in some pathological situations [39,40]. Nevertheless, some publications report their detection in labile blood products [20,21]. Many processes to which blood products are exposed may generate MPs. This is the case, for instance, of pathogen inactivation/reduction technologies [17,41], the use of conservation solutions [15], or even contact with bag [4] (Table 1). Platelet or RBC collection methods may also affect MP generation. Thus, apheresis platelets expected to be purer than platelet products obtained from whole blood *via* buffy coats or platelet-rich plasma demonstrate an increased load of PMPs or RMPs after storage [23]. Overall, the different steps involved in the preparation of labile blood products (Table 1), and especially their storage, generate MPs that may have functional consequences in transfused patients. Increased MP formation may be directly related to the "storage lesions", well-known by the transfusion community and common to both RBC and platelet concentrates. One has to mention that blood storage is responsible not only for increased MP formation, but also for the modification of MP composition over time [33], and in particular variation of phosphatidylserine (PS) expression [10,13] and more generally in the membrane lipids profile [42,43]. This is not surprising since the modifications of RBC content and surface are well known features of storage lesions [44]. In contrast, filtration of blood products may possibly reduce their MP content [11,14,27]. Before evoking the potential functions of MPs in blood products, one has also to specify that the number of MPs in the bags vary from one donor to another [12,26]. This highlights that in parallel with the development of adequate MP quantification methods, clinical studies in transfused patients and experimental models have to be performed in order to assess the correlation between the side effects of transfusion and MP content.

2.1. Potential functions of labile blood product-contained microparticles

It is now widely admitted that MPs are new players in intercellular communication. Indeed, MPs are considered as critical effectors involved in numerous biological pro-