3. Bjorck P, Axelsson B, Paulie S. Expression of CD40 and CD43 during activation of human B lymphocytes. Scand J Immunol 1991;33:211-8.

120

Scanning Flow Cytometry Study of Cell-Derived Microparticles and Their Aggregates in Platelet-Rich Plasma

Daria Chernova[1,2], Anastasiya Konokhova[1], Maxim Yurkin[1,2], Valeri Maltsev[1,2]
[1]Institute of Chemical Kinetics and Combustion SB RAS, Novosibirsk, Russia, [2]Novosibirsk State University, Novosibirsk, Russia

Introduction: Reliable detection and analysis of single cell-derived microparticles (MPs) is complicated by their small sizes and low refractive indices (RIs), that motivates development of new techniques to identify MPs in biological fluids, distinguishing them from other constituents, and to characterize MPs by their shape, size, and RI.

Methods: We developed a flow-cytometry-based method for identification and characterization of MPs in platelet-rich plasma from light scattering. Using a scanning flow cytometer we measure angle-resolved light-scattering profiles (LSPs) and side scattering for individual particles in plasma. These data is used to deduce particle size, shape, and RI from the solution of the inverse light-scattering (ILS) problem given the adequate optical model of the particle. We utilized the following light-scattering simulation methods: discrete-dipole approximation, Mie-theory, and T-matrix method for platelets, sphere-like and bisphere-like MPs respectively. Those particles were separated from other detected events, including larger MP aggregates and other non-spherical plasma constituents, based on agreement between with measured and calculated LSPs. Thus, we not only identify individual MPs among other particles, but also characterize them by their size and RI, including errors of these estimates.

Results: We analyzed a sample of platelet-rich plasma. All detected events were separated into platelet and MP events, including sphere-like, bisphere-like and not-identified particles (none of proposed models is suitable). Identified MPs were characterized by their distributions over size and RI. Polystyrene beads of 0.4 and 1 μm were also measured. The developed method and current setup of the SFC allowed us to reliably characterize the fraction of single spherical MPs falling in the range of 450–600 nm for size and 1.48–1.52 for RI, the median uncertainties of single measurements were 6 nm and 0.003, respectively. RI of beads is 1.617–1.618, which agrees with the literature data for polystyrene at 405 nm.

Conclusion: Presented light-scattering method allows one to identify single spherical MPs and biosphere-like MP aggregates in platelet-rich plasma and characterize them by their size and RI with high precision.

121

Standardization of Flow Cytometry-Based Determination of Plasma Microvesicles: Recent Progress

Philippe Poncelet[1], Romaric Lacroix[2,3], Coralie Judicone[1,3], Stéphane Robert[2], Micah Mooberry[4], Nigel Key[4], Françoise Dignat-George[2,3]
[1]Research & Technology, Biocytex, Marseille, France, [2]VRCM, UMR-S1076, Aix-Marseille University, INSERM, Marseille, France, [3]Hematology and vascular biology department, Hôpital de la Conception, APHM, Marseille, France, [4]Department of Medicine, University of North Carolina, Chapel Hill, NC, United States

Background: Although cell-derived microvesicles (MV) counts may provide useful diagnostic/prognostic information, assessment of their pathophysiological relevance in multicentre studies is hampered by methodological concerns and a lack of standardization. Aiming to define the usefulness of MV as true biomarkers in clinical practice, the Scientific Sub-Committee on Vascular Biology (VB-SSC) of the International Society of Thrombosis and Haemostasis (ISTH) has set up a network aimed at mastering pre-analytical and analytical variables (mainly FCM-related) for MV analysis. First, a pre-analytical protocol significantly reducing the variability of MV measurement was validated in a multicentre study (1). Second, a collaborative workshop using standard Flow Cytometry (sd-FCM) in 2009 defined the inter-laboratory variability of PMV counts (2). Although a specific bead-based calibration system proved to be useful for instrument qualification and monitoring, differential behavior among flow cytometer (FCMr) sub-types impeded a universal standard set-up. Third, a modified strategy has been recently proposed to provide optimized scatter-based reference levels for either FSC- or SSC-optimized FCMrs (3) and to cope with higher sensitivity FCM (hs-FCM, 4).

Aim: Based on this new approach, a 2nd collaborative workshop was initiated to evaluate inter-instrument reproducibility of MV counts among different platforms.

Methods: The 1st phase (2013) aimed at selecting FCMrs based on scatter resolution and background level. In the 2nd phase (2014), selected labs received frozen aliquots of plasma featuring various platelet MV (PMV) counts as well as common reagents. In addition to the bead-based Q.C. tools (Megamix-Plus FSC or Megamix-Plus SSC, depending on instrument), these included PMV staining reagents and 3 μm counting beads compatible with hs-FCM.

Results: From Phase 1, ~85% of n=59 candidate FCMrs featuring 14 types from 44 labs passed the MV-oriented Q.C., most of which confirmed qualification a year after. After Phase 2 of this multi-center exercise, usable data were gained from n=29 FCMrs and illustrated that comparable PMV counts can be achieved among different labs, even using different platforms. Indeed, the mean PMP counts measured in each group of FCMrs (FSC- or SSC-opimized) were not statistically different from one group to the other nor from those determined by the core-lab with CVs across all labs of 31%, 40% and 51%, depending on the sample. These CV contrast with previously observed differences in orders of magnitude.

Conclusion: The results of this international exercise for the standardized counting of PMVs with currently available FCMrs open the door for future multi-center studies. Although it should not stop ISAC's technological search for higher sensitivity, this standardization effort may already help taking the best from actual FCMrs.

1 & 2) Lacroix et al, JTH 2010 & 2011 3) Poncelet et al, CYTO 2012; 4) Robert et al, ATVB 2012.

122

Quantification of Cell-Derived Microvesicles in Blood

Alain Brisson, Nicolas Arraud, Celine Gounou, Romain Linares
UMR-CBMN, University of Bordeaux, Pessac, France

Background: Cell-derived microvesicles (MVs) play major roles in health and disease. Elevated MV levels have been found in plasma and other body fluids in many pathological disorders, leading to the hypothesis that MVs may serve as disease biomarkers. However, the small size of MVs renders difficult their characterization, principally their quantification, as attested by major discrepancies in MV concentrations reported in the literature. It is therefore critical and urgent to design reliable and sensitive methods of MV quantification. We have recently shown that a simple flow cytometry (FCM) method based on fluorescence triggering enabled to detect 50 x more Annexin-5-positive (Anx5+) MVs than conventional FCM methods based on light scatter triggering (1).