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Effects of donor age, donor sex, blood-component processing, and storage on cell-derived microparticle concentrations in routine blood-component preparation

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ABSTRACT

Background: A number of factors cause increases in the number of cell-derived microparticles (MPs) in blood components. However, the overall effects of these factors on the concentration of MPs during routine blood-component preparation have not fully been elucidated.
Aim: To evaluate the effects of donor age, donor sex, blood-component preparation, and storage on MP concentrations.
Methods: Flow cytometry was used to quantitate the number of whole blood-derived MPs.
Results: The total MP concentration was similar in male and female donors (26,044 ± 1254 particles/μL vs. 27,696 ± 1584 particles/μL). The total MP concentration did not differ significantly among the different age groups: 18–30 years (28,730 ± 1600 particles/μL), 31–40 years (24,972 ± 5947 particles/μL), and 41–58 years (25,195 ± 1727 particles/μL). However, the total number of MPs in fresh plasma (152,110 ± 46,716 particles/μL) was significantly higher ($p < 0.05$) than that in unprocessed whole blood (26,752 ± 985 particles/μL), fresh packed red blood cells (PRBCs) (28,574 ± 1028 particles/μL), and platelet concentrate (PC) (33,072 ± 1858 particles/μL). Furthermore, the total numbers of MPs in stored PRBCs and fresh-frozen plasma (FFP) were significantly higher ($p < 0.05$) than those in fresh PRBCs and fresh plasma, respectively.
Conclusions: The study suggests that donor factors, blood-component processing and storage contribute to the MP concentration in routine blood-product preparation. The findings can improve quality control and management of blood-product manufacturing in routine transfusion laboratories.

1. Introduction

The formation of cell-derived microparticles (MPs) disrupts the interaction between the cytoskeleton and lipid bilayer [1]. Upon apoptosis or cellular activation, MPs can be generated from various cells, including red blood cell-derived (RMPs), platelet-derived MPs (PMPs), leukocyte-derived MPs (LMPs), and endothelial cell-derived MPs (EMPs). Accumulated evidence has demonstrated elevated numbers of MPs in several diseases and pathological conditions [2,3]. Apart from the roles of MPs in disease, studies have documented the clinical importance of MPs in transfusion medicine [4,5]. The procoagulant activity of MPs in stored blood components has also been demonstrated [6]. In addition, recent studies suggested that elevated numbers of RMPs in blood products may be responsible for complications in blood transfusion recipients by modulating the function of immune cells [7,8] and that transfusions containing high doses of RMPs may be associated with an inflammatory response [9]. Studies also indicated that an interaction between PMPs and leukocytes may be responsible for allergic transfusion reactions [10,11]. Furthermore, research demonstrated that LMPs played a role in endothelial dysfunction by recruiting inflammatory cells to the vascular system, leading to the progression of atherosclerotic lesions [12]. Given the potential effects of MPs in post-transfusion reactions, determining the factors that contribute to MP concentrations in blood products is important to minimize MP concentrations and reduce the risks of transfusion reactions associated with MPs.

Recent studies of factors contributing to increased numbers of MPs in blood components pointed to an association between donor variability and increased numbers of MPs in blood components [13,14]. Different blood components are prepared using different procedures,

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which influence the concentration of MPs in blood products [15–17]. After preparation, some blood components may be stored before being transfused into recipients. The effect of this storage on MP concentrations has been documented. One study found a higher number of MPs in older RBCs than in fresh ones [18]. In a study of MPs in fresh-frozen plasma (FFP), both the preparation protocol and storage time were associated with increased numbers of MPs in FFP [15]. In addition to the aforementioned factors, studies demonstrated that the rate of MP accumulation in RBCs stored in citrate phosphate dextrose (CPD)/saline, adenine, glucose, and mannitol was slower than that in RBCs stored in citrate phosphate dextrose adenine (CPDA) [19]. Despite knowledge of the contribution of these factors to increased numbers of MPs in blood components, the overall effect of these factors on MP concentrations in routine blood-component preparation remains unclear.

In the current study, we compared MP levels in 1) donors of different ages and sex; 2) fresh packed RBC (PRBCs), platelet concentrate (PC), and fresh plasma versus those in unprocessed whole blood; 3) fresh PRBCs versus those in stored PRBCs; and 4) fresh plasma versus those in FFP.

2. Material and methods

Fluorescein-isothiocyanate conjugated annexin V (Annexin V-FITC), phycoerythrin-conjugated CD235a (CD235a-PE), peridinin chlorophyll-conjugated CD45 (CD45-PerCP), allophycocyanin-conjugated CD41a (CD41a-APC), and 10 × annexin V binding buffer were purchased from ImmunoTools (Friesoythe, Germany). CountBright™ counting beads were obtained from Invitrogen (Carlsbad, CA, USA). Blank calibration particles of 1.09 µm were purchased from Spherotech (Lake Forest, IL, USA).

2.1. Blood-component preparation and sample collection

The present study was approved by the Institutional Review Board of Siriraj Hospital, Mahidol University School of Medicine, Bangkok, Thailand (COA no.395/2016). Written consent was obtained from blood donors after the procedure had been explained to them in detail, including its benefits and possible hazards. Blood samples were then collected and processed using standard procedures of the Department of Transfusion Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University. Whole blood samples were collected in tubes containing tripotassium ethylenediaminetetraacetic acid, and complete blood counts (CBCs) were then analyzed using a Coulter AcT 5-part differential (5 diff) (Beckman Coulter, Fullerton, CA, USA). These samples were also used for MP analysis and defined as unprocessed whole blood.

The overall processes of blood component manufacturing and sample collection are summarized in Fig. 1. Units of whole blood were collected into triple blood collecting systems (JMS Triple Blood Bag; CPD-SAGM Solution; JMS Singapore Pte. Ltd., Singapore). Each system included a 450-ml collection bag containing 63 ml of CPD, a 400-ml bag containing 100 ml of SAGM red cell preservative solution, and a 400-ml bag for 5-day platelet storage. All units of whole blood were stored at room temperature (22 ± 2 °C) for up to 8 h before the blood components were prepared. The units of whole blood were centrifuged at 3100 × *g* for 5 min at 22 ± 2 °C in a centrifuge (Heraeus™ Cryofuge™ 6000i; Thermo Electron LED GmbH, Langenselbold, Germany) and separated into PRBCs and platelet-rich plasma using a manual extractor. The platelet-rich plasma was transferred into a 400-ml bag for platelet storage, centrifuged at 3800 × *g* for 5 min at 22 ± 2 °C, and separated into platelet-poor plasma and PC. After the addition of 100 ml of SAGM, the units of PRBCs were stored at 4 ± 2 °C. The plasma units were then transferred into a 400-ml bag, rapid-frozen to -30 °C using a shock freezer (TPSU 40; Thalheimer, Ellwangen, Germany or MBF 12; Dometic, Hosingen, Luxembourg) and stored at -30 ± 10 °C. The FFP was thawed in a plastic bag in a water bath (Memmert Waterbath WPE45; Memmert GmbH + Co. KG, Germany) or a plasma thawer (W-PFD® Plasma Fast Thawer; KW Apparecchi Scientifici, Italy) for 20–30 min at 37 °C. The samples were processed to quantitate MPs within 2 h after finishing the preparation process and before sending each blood component to its recipient for transfusion.

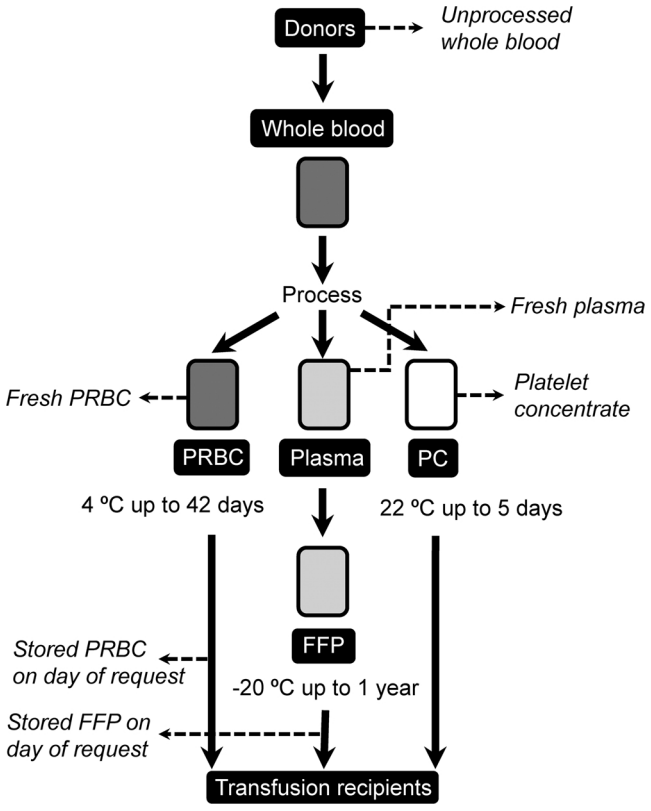

Fig. 1. Schematic diagram showing blood component processing (solid arrows) and sample collection (dotted arrows).

PRBC, plasma, platelet concentrate, and FFP samples were taken from heat-sealed segments after thorough mixing of the bag, followed by stripping of the sampling pipe. The content of each segment was immediately transferred into a 0.5 mL microcentrifuge tube and carefully mixed before determination of the MP concentration.

2.2. Flow cytometry quantitation of MPs

The whole blood samples, PRBCs, and PC were diluted with phosphate-buffered saline (1:100). Undiluted FFP samples were used for MP analysis. For analysis, 5 µL of each sample was incubated with 3 µL each of annexin V-FITC, CD45-PerCP, and CD41a-APC; 2 µL of CD235a-PE; and 20 µL of 1 × annexin V binding buffer. After 15 min, 300 µL of 1 × annexin V binding buffer was added to the tubes. The samples were analyzed immediately using an FACSCalibur flow cytometer (BD, San Jose, CA, USA) equipped with two lasers: a 488 nm (blue) and 635 nm (red).

The MP gate was set on the dot-plot of forward scatter versus the side scatter (SSC) according to 1-µm standard beads. The SSC versus the annexin dot-plot was used to identify the total number of annexin V-positive MPs (Fig. 2). Then, the events of RMPs, PMPs and LMPs from the previous dated annexin V-positive MPs were determined on a histogram plot of CD235a-PE, CD41a-APC, and CD45-PerCP, respectively. The MP concentrations were quantitated as described previously [20]. The consistency of the flow rate was monitored throughout the acquisition period. Prior to a dilution experiment, the performance of the flow cytometer was optimized using CaliBRITE beads (BD, San Jose, CA, USA) and FACSComp software, version 5.1. The dilution