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Influence of erythrocyte aggregation on radial migration of platelet-sized spherical particles in shear flow

Cyrille Guilbert [a,1], Boris Chayer [a], Louise Allard [a], François T.H. Yu [a,2], Guy Cloutier [a,b,c,*]

[a] Laboratory of Biorheology and Medical Ultrasonics, University of Montreal Hospital Research Center (CRCHUM), Montréal, Québec, Canada
[b] Institute of Biomedical Engineering, University of Montreal, Montréal, Québec, Canada
[c] Department of Radiology, Radio-oncology and Nuclear Medicine, University of Montreal, Montréal, Québec, Canada

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ABSTRACT

Blood platelets when activated are involved in the mechanisms of hemostasis and thrombosis, and their migration toward injured vascular endothelium necessitates interaction with red blood cells (RBCs). Rheology co-factors such as a high hematocrit and a high shear rate are known to promote platelet mass transport toward the vessel wall. Hemodynamic conditions promoting RBC aggregation may also favor platelet migration, particularly in the venous system at low shear rates. The aim of this study was to confirm experimentally the impact of RBC aggregation on platelet-sized micro particle migration in a Couette flow apparatus. Biotin coated micro particles were mixed with saline or blood with different aggregation tendencies, at two shear rates of 2 and 10 s^{-1} and three hematocrits ranging from 20 to 60%. Streptavidin membranes were respectively positioned on the Couette static and rotating cylinders upon which the number of adhered fluorescent particles was quantified. The platelet-sized particle adhesion on both walls was progressively enhanced by increasing the hematocrit ($p < 0.001$), reducing the shear rate ($p < 0.001$), and rising the aggregation of RBCs ($p < 0.001$). Particle count was minimum on the stationary cylinder when suspended in saline at 2 s^{-1} (57 ± 33), and maximum on the rotating cylinder at 60% hematocrit, 2 s^{-1} and the maximum dextran-induced RBC aggregation (2840 ± 152). This fundamental study is confirming recent hypotheses on the role of RBC aggregation on venous thrombosis, and may guide molecular imaging protocols requiring injecting active labeled micro particles in the venous flow system to probe human diseases.

1. Introduction

Platelets play major roles in multiple mechanisms of hemostasis and thrombotic events. They intervene in repairing vascular injury to prevent further blood lost or pathologically to induce thrombus growth. Therefore, platelets must leave the main blood stream and adhere to the vascular endothelium of damaged or inflamed sites. Red blood cells (RBCs), because of their size, their aptitude to deform as well as their important volume fraction in blood, facilitate transport of platelets within blood vessels (Aarts et al., 1983, 1984; Eckstein et al., 1988; Turitto and Hall, 1998; Zhao et al., 2012; Reasor et al., 2013; Litvinov and Weisel, 2017).

A contemporary review of blood margination (*i.e.*, radial displacement of leukocytes and platelets toward the wall) and segregation (*i.e.*, spatial redistribution of blood elements) phenomena can be found in Kumar and Graham (2012).

1.1. Mass transport

Transport of matter in a fluid can be done by convection (due to the principal flux) and/or diffusion (due to chaotic movement). *In vitro*, adhesion of platelets to the vascular wall was almost abolished in the absence of human RBCs because platelets travelled parallel to streamlines due to convection only (Aarts et al., 1983). Similar behavior was observed *in vivo*, the presence of RBCs had a strong impact on micro particle margination in microvessels (D'Apolito et al., 2015). The presence of RBCs would promote particle diffusion through a *turbulent mixing effect* (Keller, 1971). Deformable particles, such as RBCs in shear flow, exhibit a steady stationary-orientation motion or an unsteady flipping motion (Keller and Skalak, 1982; Barthes-Biesel and Sgaier, 1985) that

* Corresponding author at: Laboratory of Biorheology and Medical Ultrasonics, University of Montreal Hospital Research Center (CRCHUM), Montréal, Québec, Canada.
E-mail address: guy.cloutier@umontreal.ca (G. Cloutier).
[1] Now with the Centre Hospitalier de la Côte Basque (CHCB), Bayonne, France.
[2] Now with the Center for Ultrasound Molecular Imaging and Therapeutics, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.

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contribute to platelet margination. RBCs migrate toward the center of blood vessels and their deformability expulses the smaller platelets from the central flow toward the vessel wall. Through this mechanism, a two-phase region is anticipated: a region rich in RBCs and poor in platelets at the vessel center, and a region rich in platelets and poor in RBCs at the periphery. Diffusive transport is provoked by the constant collisions of suspended particles. Since there are more particles per unit volume in the central region of a blood vessel, platelets tend to migrate from the interior of the vessel to the vascular wall, as observed experimentally by laser Doppler (Aarts et al., 1988).

The rotation of RBCs in shear flow has a *swept-out volume* whose amplitude depends on the RBC deformability (Chien, 1975). The more the RBC deforms, the less the swept out volume is important. Goldsmith (1972) proposed that RBCs undergo an erratic radial displacement resulting from constant collisions between suspended particles, thus provoking the radial diffusion of platelets. With this theory, the rate at which platelets are transported is proportional to the shear rate and the hematocrit but not to the diameter of the RBC. The enhancement of micro particle migration with shear rate was experimentally observed in a microfluidics system at shear rates between 800 and 2500 s^{-1}, when interacting with 10% hematocrit RBCs (D'Apolito et al., 2016). The presence of a *skimming layer* (Aarts et al., 1983) could constitute another explanation for the platelet diffusion phenomenon. This effect is attributed to the important size difference between RBCs and platelets. Interestingly, both theories lead to the same conclusion that RBCs favor platelet migration toward the vessel wall.

1.2. Effect of RBC volume concentration

The presence of RBCs at 40–50% hematocrit considerably contributes to the displacement of platelets and leukocytes, and their adhesion on the endothelium surface (Goldsmith et al., 1999). At such a high volume concentration, one might think that RBCs would limit the diffusion of suspended particles. This is only true under plug flow when moving blood is stationary with no streamline mixing (Goldsmith and Turitto, 1986). At the opposite, the presence of a velocity gradient promotes collisions of cells approaching on adjacent streamlines. By observing the movement of tracer particles in a suspension of transparent ghost RBCs (*i.e.*, hemoglobin-less RBCs) under laminar flow using a travelling microscope, several findings on cell kinetics have been reported (Goldsmith, 1993): (1) In the absence of ghost erythrocytes, tracer particles travelled undisturbed and parallel to the vascular wall; (2) when ghost RBCs were added, cellular collisions increased and a random lateral displacement of smaller spherical tracer particles was noticed (Goldsmith and Marlow, 1979); and (3) collisions with the vascular wall occurred and the frequency of collision increased with the ghost RBC volume concentration. Recent simulations of microcirculatory flow at 24% hematocrit also reported that the frequency of collisions with RBCs favors micro particle margination (Vahidkhah and Bagchi, 2015).

1.3. Influence of RBC aggregation

Inflammatory conditions promote abnormal RBC hyper-aggregation (Weng et al., 1996a, 1998; Ben Ami et al., 2001; Berliner et al., 2005; Maharskak et al., 2009; Reggiori et al., 2009; Tripette et al., 2013). The hyperviscosity provoked by this phenomenon increases the interaction time of platelets with the vascular wall and favors venous thrombotic events (Yu et al., 2011). However, the decisive role of RBC aggregation on platelet radial migration is still underexplored. Nash et al. (2008) proposed that even though RBC aggregation is not required for the margination of platelets, its presence would favor it. Opposite results were, however, obtained *in vivo* using the intravital microscopy technique and fluorescently labeled platelets flowing in rabbit mesenteric arterioles and venules. RBC hyper-aggregation induced by high-molecular weight dextran infusion reduced the platelet count at the wall (Woldhuis et al., 1993). Using the same technique, RBC aggregation promoted leukocyte margination in rat postcapillary venules and their adhesion on the endothelium (Pearson and Lipowsky, 2000). The enhancement of leukocyte margination with RBC aggregation has also been demonstrated in a rectangular channel microfluidic system (Jain and Munn, 2009).

1.4. Objective

The current study aimed to quantify experimentally in a Couette flow device the radial migration of platelet-sized micro particles as a function of RBC aggregation, flow shear rate and blood hematocrit. RBC aggregation is a recognized thrombotic co-factor (Mackman, 2012) that has been postulated to trigger venous thrombosis (Yu et al., 2011). The current study proves the direct impact of RBC aggregation on mimicking platelet transport, which is required for interaction with the vessel wall during venous thrombosis. This work may also shed some light and guide molecular imaging protocols based on the injection of micro particles into the venous system (Katoh et al., 2009). Experimental data are also required to better understand the interaction of drug micro carriers with blood flow properties (Müller et al., 2014), and the current study may provide new evidences.

2. Materials and methods

2.1. Experimental considerations

Platelets play an important role in the mechanisms of thrombosis and hemostatic plug despite the fact that they occupy only ~0.7% of the blood cellular phase volume. This is due to their very strong reactivity once activated. Platelets manipulated outside the human body coagulate very quickly on synthetic surfaces. It is therefore very difficult to work with blood platelets without anticoagulation (Schneider et al., 1997). However, anticoagulation inhibits adenosine diphosphate induced platelet activation and, therefore, platelet adhesion. Thus, a protocol aiming at determining the impact of RBC aggregation on platelet interaction with a vessel wall would be difficult with native anticoagulated platelets. Because this study does not address the biology but the rheology of blood platelets, we propose to replace them with micro particles that have similar physical dimensions and controllable adhesion properties. Since physiological flow in blood vessels implies a spatial variation of the shear rate contributing to the micro particle margination process, we opted instead for a Couette flow system with an aspect ratio providing a close to constant shear rate in the gap between rotating cylinders (Whorlow, 1980). With such device, the confounding impact of the radial shear rate gradient on RBC aggregation did not have to be considered when interpreting our results.

2.2. Preparation of blood samples

Fresh porcine whole blood, anticoagulated with 3 g/L of ethylene diamine tetra acetic acid (EDTA ACS reagent, 99.4%, Sigma Chemical, St. Louis, MO, USA), was obtained from a local slaughter house. The buffy coat was removed after a first centrifugation at 2500 rotations per minute during 15 min. The plasma was separated from the RBCs and replaced with an isotonic saline solution for a second centrifugation. The saline suspension was aspirated to remove residual platelets and white blood cells. RBCs were resuspended in native plasma or in a saline solution.

Nine blood aliquots of 70 mL were prepared from every porcine blood sample by adjusting the hematocrit to 20%, 40% and 60%, and the RBC aggregation level into 3 categories: no aggregation (*NA*), normal aggregation (*A*) and hyper-aggregation (*HA*). *NA* samples were obtained by suspending RBCs into physiological isotonic saline. Normal *A* samples were obtained by reconstituting RBCs with the native plasma (porcine RBCs have a similar aggregation tendency as human RBCs (Weng et al., 1996b)). *HA* samples were prepared by mixing RBCs with a dextran solution. Dextran powder (512 kDa, D5251, lot 124H0055, Sigma Chemical, St. Louis, MO, USA) was dissolved into isotonic saline at a concentration of 30 g/L (Boynard and Lelievre, 1990; Neu and Meiselman, 2002). Five experimental series were realized for every blood sample at a given shear rate and hematocrit. All experiments were conducted within 48 h after blood collection. Two control solutions (no RBCs) were tested to verify the degree of micro particle migration in the absence of erythro-

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