Human monocytes from fresh blood present strong magnetic characteristics; CTV analysis and magnetic susceptibility quantification

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ABSTRACT

The presence of iron in circulating monocytes is well known as they play essential roles in iron recycling. Also, the storage of this metal as well as its incorrect uptake and/or release are important data to diagnose different pathologies. It has been demonstrated that iron storages in human blood cells can be measured through their magnetic behavior with high accuracy, however, the magnetic characteristics of monocytes have not been reported so far, to the best of our knowledge. Therefore, in this work, we report, for the first time, the physical and magnetic properties of human monocytes, along with plasma platelets, oxyhemoglobin red blood cells (oxyHb-RBCs) and methemoglobin red blood cells (metHb-RBCs). The different cell populations were separated by Ficoll-density gradient centrifugation, followed by a flow sorting step to isolate monocytes from peripheral blood mononuclear cells. The different fractions were analyzed by Coulter Counter (for determining the size distribution and concentration) and the sorted monocytes were qualitatively analyzed on ImageStream, a state-of-the-art imaging cytometer. The analysis of the Coulter Counter and ImageStream data suggests that although there exists contamination in the monocyte fraction, the integrity of the sorted monocytes appears to be intact and the concentration was high enough to precisely measure their magnetic velocity by Cell Tracking Velocimetry (CTV). Surprisingly, monocytes reported the highest magnetic mobility from the four fractions under analysis, with an average magnetic velocity 7.8 times higher than MetHb-RBCs, which is the only type of cells with positive magnetic velocities. This value is equivalent to a susceptibility 2.5 times higher than the value reported by fresh MetHb-RBCs. It should be noted that this is the first study that reports that a subpopulation of human monocytes is much more magnetic than MetHb-RBCs, opening the door to the possible isolation of human monocytes by label-free magnetic techniques.

Keywords: monocytes, platelets, RBCs, magnetic susceptibility, cell tracking velocimetry, fluorescent activated cell sorting.

1. INTRODUCTION

Monocytes are circulating blood cells that account for 2–8% of leukocytes. They play important roles in the inflammatory response and they are also able to phagocytose pathogens and damaged cells (1). Thus, isolation of monocytes from complex media such as blood has a wide range of applications, including the potential to provide insight into various normal and abnormal cell recycling. Whereas positive and negative magnetic separation techniques have been developed for monocyte isolation, little information is available regarding the intrinsic magnetic properties of these cells. However, multiple studies have demonstrated the presence of iron within monocytes, and this fact may imply that they are not as diamagnetic as most cells have typically been assumed. In fact, monocytes are involved in the recycling of iron, and hence, they play central roles in iron homeostasis. Monocytes/macrophages have evoked different pathways by which they can acquire iron, including transferrin mediated iron uptake, transmembrane uptake of ferrous and possibly also of ferric iron, iron acquisition via lactoferrin receptors, ferritin receptors or via erythrophagocytosis (2,3). Iron uptake, storage and release by monocytes are regulated at multiple steps and these regulation mechanisms can be affected by different diseases. Therefore, iron storage within monocytes or its incorrect uptake and/or release are important data to further evaluate different diseases.

One possibility method to analyze the iron content inside a cell, and in this case a monocyte, is the measurement of the cell's magnetic susceptibility (4). Over the years, we have been developing a way to quantitatively characterize the magnetic behavior of biological entities with an instrument referred to as Cell Tracking Velocimetry (CTV). In this study, we describe for the first time the magnetic behavior of human monocytes, by observing their motion when they are exposed to an external magnetic field. Furthermore, plasma platelets, oxyhemoglobin red blood cells (oxyHb-RBCs) and methemoglobin red blood cells (metHb-RBCs) are also analyzed as a reference/comparative data. Finally, their physical characteristics are also studied through state-of-the-art imaging cytometer.

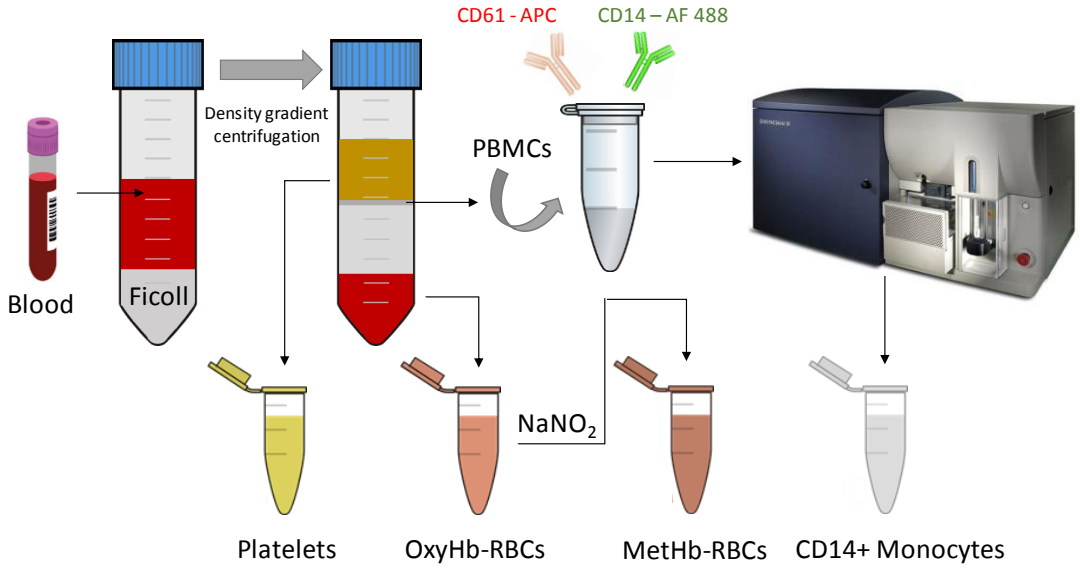

Figure 1. Workflow of the sample preparation procedure. First, human fresh whole blood was separated into three fractions by density gradient centrifugation: (i) platelets,(ii) RBCs (oxyhemoglobin RBCs), and (iii) PBMCs. The PBMCs were further processed by FACS targeting for monocytes. MetHb-RBCs were obtained by treating the OxyHb-RBCs with $NaNO_2$.

2. MATERIALS AND METHODS

Human whole blood from ten healthy subjects was collected with informed consents according to a protocol approved by the Institutional Review Board (IRB) from The Ohio State University. Approximately 15 mL of blood was drawn and collected into two 10 mL tubes containing EDTA anticoagulant. Then, using Ficoll – Paque PREMIUM density gradient media, 12 mL of the drawn-out blood was separated into four populations following the manufacturer's instructions: i) RBCs (accumulated at the bottom of the centrifugation tube); ii) Ficoll media, which was later discarded; iii) peripheral blood mononuclear cells (PBMCs), which can be distinguished as a grayish colored ring over the Ficoll media and contain the monocytes; and iv) plasma layer containing platelets.

RBCs were divided into two aliquots designated "metHb-RBCs" and "OxyHb-RBCs". The RBCs were collected from the bottom of the centrifugation tube and washed with PBS and were left open in the room for 10 minutes to ensure they were in oxyHb state. The MetHb-RBCs were obtained after treating the washed-RBCs with $NaNO_2$. PBMCs were collected after performing the density gradient centrifugation, washed with PBS, and incubated with Alexa Fluor® 488 Mouse Anti-Human CD14. The incubated samples were loaded onto flow cytometer BD FACS ARIA III and the CD14+ population was collected as shown in Fig. 1. The sort was operated until approximately 200,000 events were collected. The viability of the CD14+ monocytes was assessed by their analysis on an imaging cytometer, ImageStreamX, were 488 positive samples' images were recorded. Finally, platelets were processed as collected from the top plasma layer after centrifugation with the Ficoll – Paque PREMIUM density gradient media.

The concentration and size distribution of the different cell populations were characterized using B23005 Multisizer 4e Coulter Counter before further processing. After these analyses were performed, the samples were loaded onto the CTV. The CTV instrument configuration reported in this study has been reported in our previous publications (4,5). Basically, the different cell populations were loaded into a glass channel (each fraction had approximately the same volume of 1 ml), where they are subjected to a known magnetic field and gradient. Approximately 20 s were taken to wait for the fluid environment in the channel to reach steady state. After the samples began to settle, images of the samples' movement were captured (50 video images, 1 s interval) and further processed using in-house analysis program for calculation of the cell mobilities. More specifically, the CTV characterizes both the magnetically induced velocity, and gravity induced settling velocity of the cells, using a combination of computer tracking algorithms of microscopic images captured with a charged couple device, CCD camera. The camera captures the movement of the samples in the region of interest (ROI); within this ROI, a high, and well characterized, magnetic energy gradient in the horizontal direction is created and a nearly zero magnetic energy gradient in the vertical direction. Thus, when a cell is placed in this ROI, experimentally measured horizontal and vertical velocities are obtained, that can be related to specific cellular and field properties. All experiments were conducted at room temperature

3. RESULTS AND DISCUSSION

3.1 Size analysis and cell viability studies

The Ficoll density gradient separated entities were analyzed with the Coulter Counter for size-distribution characterization. The results are presented in Fig. 2 a). The size distribution of Ficoll-separated RBCs and plasma platelets match with the literature data (RBCs: 4–6 μm, platelets 2–3 μm). The PBMCs fraction appears to have different subpopulations which match the reported diameter values of platelets, lymphocytes (7 μm), and monocytes (8.79 μm) (6).

To further enrich for the monocyte population from the PBMCs, we included a FACS step to isolate the monocytes from platelets and lymphocytes. Pure monocyte samples were obtained for all the donors by targeting CD14+ cells, and platelet contamination within the sorted monocytes was also assessed by incubating the sample with platelet antibodies (APC Mouse Anti-Human CD61). After sorting and collecting the CD14+ samples (monocytes), these were qualitatively analyzed on ImageStream. The equipment was loaded with a small aliquot of the CD14+ fraction and the samples were run until at least 500 pictures were taken from individual cells/complexes. As presented in Fig. 2 b), the monocytes were attached to other cells/entities, smaller in size, that were not fluorescent in comparison to the stained monocytes (seen in green in the FITC channel). However, the cellular integrity for these monocytes appears to be intact after sorting. Moreover, the concentration of cells within the sorted samples was high enough to successfully analyze them on the CTV, along with the platelets and the two types of RBCs, to evaluate their magnetic velocity.

3.2 CTV analysis

The magnetic/settling velocities of the four cell fractions was determined using our CTV apparatus. In Fig. 3, we schematized the CTV instrument and we present the trajectories of the 4 populations under study inside the CTV for 50 s and for one of the donors. The vertical trajectories for the platelets and OxyHb-RBCs are consistent with our previous publications and indicate very low magnetic mobility (i.e., diamagnetic). In contrast, MetHb-RBCs are slightly deflected in diagonal direction inside the ROI, that is, they are deflected toward the right direction, which is consistent with a positive magnetic force acting on them due to their paramagnetic behavior. However, the CTV analysis of monocytes implied the presence of two population among monocytes, a very strong magnetic population and nonmagnetic population. In fact, the trajectories followed by this magnetic subfraction displays a very high magnetic velocity as they move almost horizontally from left to right within the ROI. Therefore, fresh human monocytes reported the highest magnetic mobility from the four fractions under analysis, with an average magnetic velocity 7.8 times higher than MetHb-RBCs, which is the only type of cells with positive magnetic velocities. This value is equivalent to a susceptibility 2.5 times higher than the value reported by fresh MetHb-RBCs. Finally, it should be noted that positive magnetic velocities were also observed for some of the plasma samples, which could be due to monocyte contamination, as both layers are in contact after the Ficoll density gradient separation was performed, and it is difficult to isolate both types of cells with high selectivity.

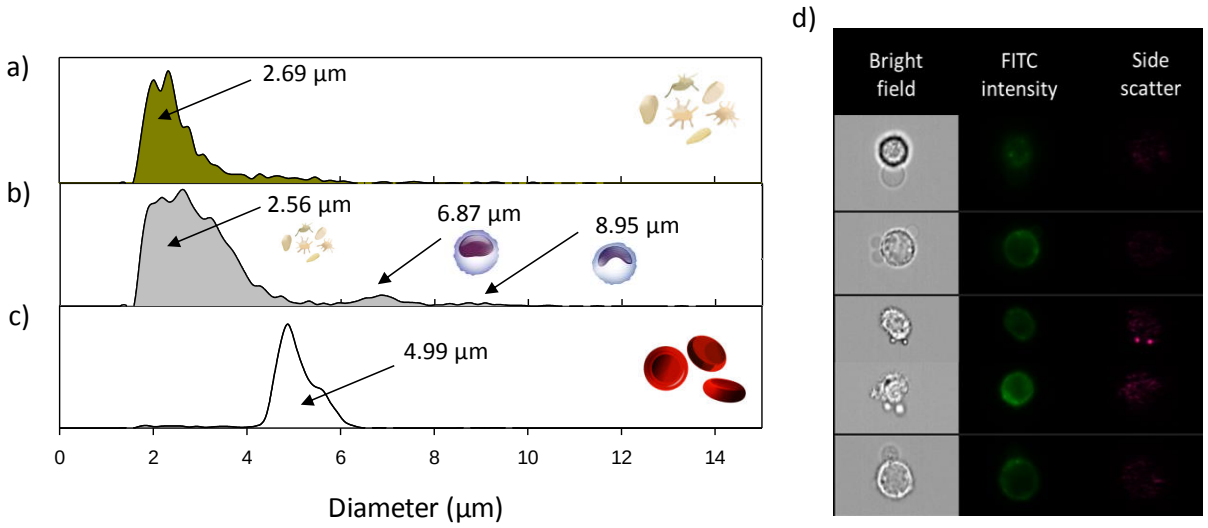

Figure 2. The histogram of size distribution of the Ficoll-separated entities characterized by Coulter Counter: a) Plasma; b) PBMCs; c) RBCs. d) Images taken from the CD14+ fraction after sorting on ImageStream, showing bright field images, FITC fluorescence intensity and side scatter.

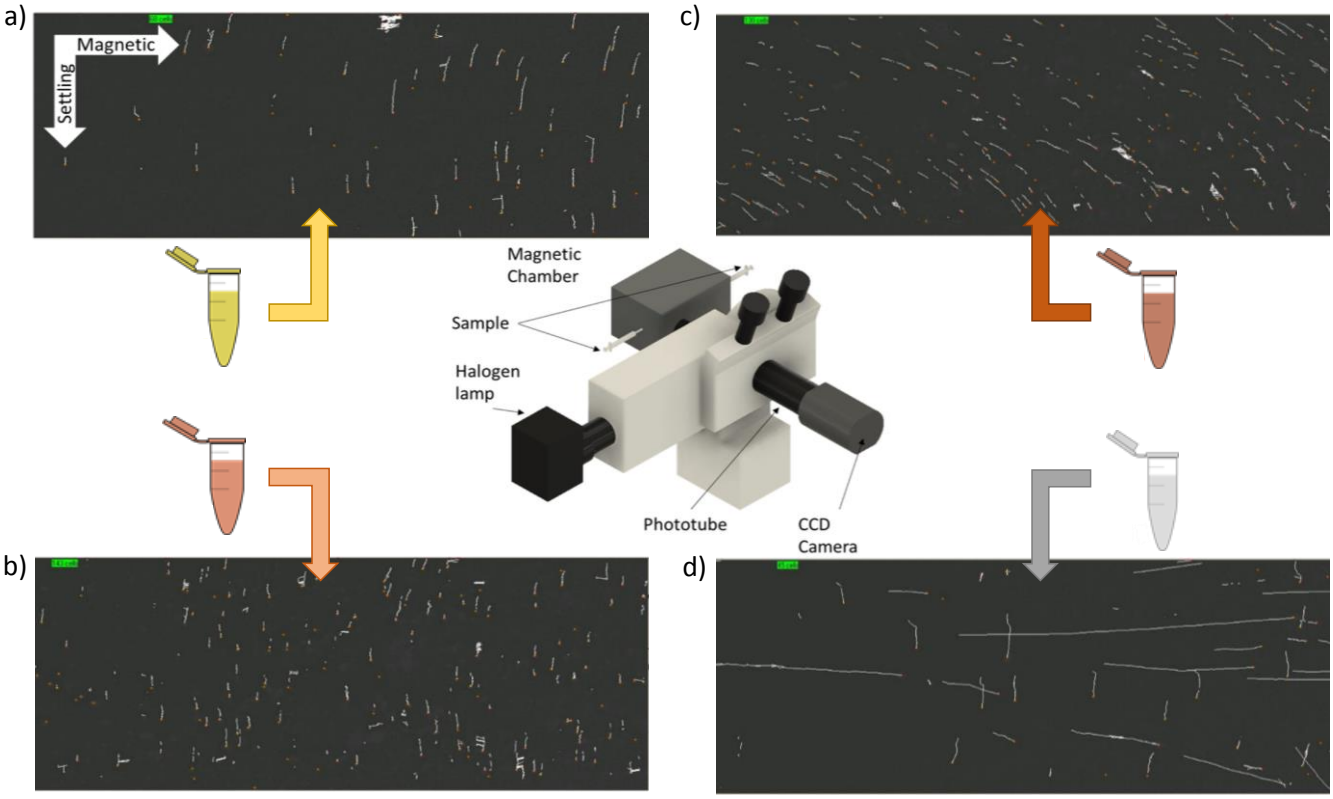

Figure 3. Schematic representation of the CTV instrument showing also the trajectories followed by blood cells within the CTV in 50 s. a) Platelets; b) OxyHb-RBCs; c) MetHb-RBCs; d) monocytes.

4. CONCLUSIONS

In this study, we have reported the magnetic analysis of human fresh blood cells with an emphasis on monocytes. Monocytes contain iron as they take part in iron recycling, and the measurement of this metal could be useful to diagnose different pathologies. Thus, we have measured here their magnetic behavior in order to asses for their iron content. We isolated monocytes from human fresh whole blood and characterized their physical and magnetic properties, along with plasma platelets, oxyHb-RBCs and metHb-RBCs. The different cell populations were separated by Ficoll-density gradient centrifugation, followed by a FACS step to isolate CD14+ monocytes. Although we observed some contamination within the monocyte fraction, their integrity was intact. The magnetic velocity and settling velocity for the four fractions was measured by tracking the cell trajectories inside the CTV apparatus. Whereas all the cell types reported similar settling velocities, the magnetic velocities varied depending on the cell population analyzed. Surprisingly, monocytes reported the highest magnetic mobility from the four fractions under analysis, with an average magnetic velocity 7.8 times higher than MetHb-RBCs. This is the first study that reports that a subpopulation of human monocytes is much more magnetic than MetHb-RBCs, opening the door to the possible isolation of human monocytes by label-free magnetic techniques. Also, this article reports the risk of using negative magnetic isolation techniques for monocytes, which will result in the loss of monocytes.

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