On the Effect of Hematocrit on Dielectric Blood Coagulometry Measurements

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***Abstract*—Dielectric blood coagulometry is a newly emerging approach for the assessment of the hemostatic potential of blood using the electronic technique of dielectric spectroscopy. Since the aggregation and deformation of red blood cells (RBCs) – two critical processes underlying blood coagulation – play a pivotal role in this approach, it is essential to characterize such measurements at reduced hematocrits for applications that involve anemic blood samples. To that end, we develop a protocol for creating reconstituted whole blood (rWB) samples with diminished hematocrits as low as ~10%. These samples are next evaluated using our microfluidic dielectric coagulometer – termed ClotChip – as well as rotational thromboelastometry (ROTEM), which is the clinical standard for viscoelastic coagulometry. We find that rWB samples with hematocrits as low as ~10% still exhibit a characteristic dispersion region at MHz frequencies that is attributed to the interfacial polarization of RBC membranes. Furthermore, we show that the two ClotChip readout parameters indicative of clotting time and clot firmness correlate well with those measured by ROTEM for rWB samples with hematocrits in the range of ~10–40%. This work illustrates the viability of ClotChip as a dielectric blood coagulometer to assess hemostatic function at hematocrits as low as ~10%.**

***Index Terms*–Dielectric coagulometry, dielectric spectroscopy, hematocrit, microfluidics, red blood cell, whole blood coagulation.**

I. Introduction

Hemostasis is a delicately balanced physiological process that serves to maintain blood flow and prevent blood loss in the event of an injury. Of the primary blood components, platelets and blood plasma are traditionally considered to serve critical hemostatic function, while red blood cells (RBCs) serve a more passive role. Nonetheless, RBCs are entrapped during fibrin clot formation and subsequently deformed during clot retraction due to contractile forces applied by activated platelets. Using our previously reported microfluidic dielectric coagulometer – termed ClotChip – we can leverage the two critical processes of RBC aggregation and deformation within a clotting blood sample to provide a comprehensive hemostatic profile of whole blood coagulability at the point-of-care using μL-volume samples [1]-[6]. This is accomplished by measuring the temporal variation in the normalized real part of blood dielectric permittivity at MHz-range frequencies [7], [8].

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Given the critical role of the RBCs in dielectric coagulometry approach, it is essential to assess the effect of hematocrit (i.e., volume fraction of RBCs in blood) on such measurements to establish whether an assay such as the ClotChip can still provide a readout that is truly reflective of a patient's hemostatic profile at diminished hematocrits. This is especially relevant for cases of genetic anemia, chemotherapy-induced anemia, or traumatic hemorrhage in which patients may present with abnormally low hematocrits. In this work, we aim to elucidate such effects and to demonstrate that ClotChip still provides a reliable hemostatic profile even at low RBC counts.

II. Study Design & Methods

A normal hematocrit lies in the range of 40–54% for adult males and 36–48% for adult females [9]. In severe cases of anemia, a patient's hematocrit can fall below 20% [10]. For this reason, we created samples with target hematocrits ranging from 10–40% to be assessed with ClotChip. Samples with reduced hematocrits can be obtained by diluting blood in saline; however, this approach would also dilute plasma proteins and platelets critical to hemostatic function. Therefore, we employed a protocol to reduce hematocrits *without* diluting the entire sample, thus maintaining platelet counts and coagulation factor concentrations in the plasma.

A. Sample preparation

De-identified, healthy, human whole blood samples in 3.2% sodium-citrate anticoagulant were obtained from the Case Western Reserve University Hematopoietic Biorepository and Cellular Therapy Core. Samples were inverted ten times to ensure an even mixing before platelet and RBC counts were obtained using a DXH500 hematology analyzer. Serial centrifugation was used to separate the blood into its constituent components (see Fig. 1.) First, samples were separated into RBC and platelet-rich-plasma (PRP) components via centrifugation at 150 g for 15 min. The RBC pellet was re-suspended in an equal volume of phosphate-buffered saline (PBS) and centrifuged at 500 g for 5 min to isolate washed RBCs from platelets, white blood cells, and plasma. The wash step was repeated three times. A portion of the PRP was centrifuged at 13,000 g for 5 min to isolate a platelet-free-plasma (PFP) supernatant. The DXH500 hematology analyzer was used to obtain platelet and RBC

counts on all three components (washed RBCs, PRP, and PFP). Based on these counts, the components were recombined into reconstituted whole blood (rWB) samples with varying hematocrits, but unmodified platelet counts and plasma protein concentrations. We targeted hematocrits of 25%, 50%, 75%, or 100% of each healthy donor's initial hematocrit. The rWB samples were measured a final time using the DXH500 hematology analyzer to verify successful sample preparation. Samples with <50K platelets/μL or >25% deviation from target hematocrits were excluded.

B. ClotChip and ROTEM measurements

The coagulation profile of the rWB samples with varying hematocrits was measured with the ClotChip and rotational thromboelastometry (ROTEM) assays in parallel. For ClotChip, 200 μL of the sample was pre-warmed at 37°C for at least 10 min. To initiate coagulation, the sample was re-calcified by adding 8.5 μL of 250-mM $CaCl_2$ for every 50 μL of blood plasma in the sample. Immediately after re-calcification, 9 μL of the sample was injected into the ClotChip, which was held at 37°C for the 30-min test duration. The dielectric permittivity of the sample was then measured at 1 MHz by an Agilent impedance analyzer as the sample underwent coagulation. The permittivity was also measured from 10 kHz–100 MHz to verify the presence of the dispersion region at MHz frequencies due to interfacial polarization of RBC membranes. Duplicate measurements for each sample were performed by the ClotChip.

To validate our ClotChip results, rWB samples were also measured using the NATEM modality of a ROTEM assay. ROTEM is the current benchmark for coagulometry and provides a viscoelastic hemostatic profile of a sample via direct contact with clotting blood. In ROTEM, a rotating pin suspended on a torsional wire is submerged into a cup containing re-calcified whole blood. As the blood coagulates, it resists the rotation of the pin. The displacement of the pin relative to the torsional wire is measured optically and is shown to relate to clotting events. The clotting time, *CT*, and maximum clot firmness, *MCF*, parameters of ROTEM have been previously shown to exhibit a strong positive correlation with the ClotChip T_{peak} and $\Delta\varepsilon_{r,max}$ parameters, respectively, at normal hematocrits [6].

To assess the rWB samples in ROTEM, 300 μL of the sample was warmed to 37°C and added to a ROTEM cup containing the appropriate amount of re-calcification buffer (i.e., 8.5 μL of 250-mM $CaCl_2$ for every 50 μL of plasma). Immediately after re-calcification, the cup was introduced to the rotating pin and a NATEM measurement was initiated. All pipetting steps were performed using the ROTEM automated pipette. ClotChip and ROTEM tests were performed within 2 hours of blood collection.

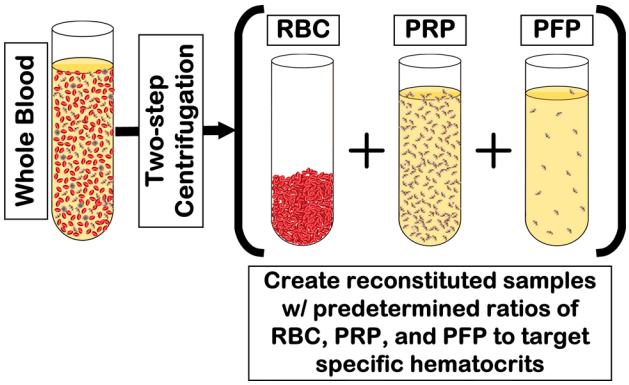

Fig. 1. Illustration of the steps involved in creating rWB samples with varying hematocrits *ex vivo*.

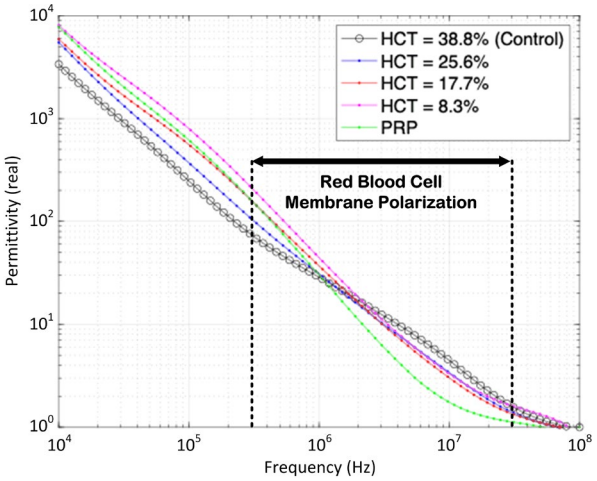

Fig. 2. ClotChip measurements showing the real part of dielectric permittivity versus frequency (10 kHz–100 MHz) for rWB samples with varying hematocrits. The permittivity curves exhibited a dispersion region due to RBC membrane interfacial polarization as evident by the absence of this region in the same measurements conducted with PRP. The dispersion region was more pronounced with higher hematocrits.

III. Measurement Results

A. Sample preparation

To verify the utility of our sample-preparation protocol, cell counts of the final rWB samples were obtained and compared to target values. To that end, we primarily focused on the RBC count as the variable-of-interest; however, we also ensured the presence of a sufficient number of platelets to yield normal clotting behavior. All rWB samples contained >50K platelets/μL, with most samples containing 100K–250K platelets/μL, quantities that fell within a normal range for platelet count. Target hematocrits were achieved with an accuracy of ±9% across all samples.

B. Variation of ClotChip and ROTEM parameters with hematocrit

The principle of dielectric coagulometry for assessment of hemostasis relies on the formation of a characteristic dispersion region at MHz frequencies due to interfacial

polarization of RBC membranes [11]. Figure 2 depicts ClotChip measurements showing the real part of dielectric permittivity versus frequency (10 kHz–100 MHz) for rWB samples with varying hematocrits. As expected, the permittivity curves exhibited a dispersion region, even with hematocrit less than 10%, as evident by the absence of this region in the same measurements conducted with PRP that lacked RBCs. The dispersion region was more pronounced with higher hematocrits.

To determine whether the dispersive effect of RBCs at low hematocrits was sufficient to characterize hemostatic profile, we extracted two parameters, namely, T_{peak} and $\Delta\varepsilon_{r,max}$, from the ClotChip readout curve defined as the temporal variation of the normalized real part of blood dielectric permittivity at 1 MHz [6]. These parameters have been previously shown to indicate the coagulation time of the sample and the strength of fibrin clot formation, respectively [6]. Similarly, the corresponding ROTEM parameters, namely, *CT* and *MCF*, were also extracted.

Figure 3 shows the variation of ClotChip and ROTEM parameters with different hematocrits. As can be seen, both ROTEM *MCF* and ClotChip $\Delta\varepsilon_{r,max}$ parameters increased with decreasing hematocrits, although the increase was not statistically significant for the latter. Notably, the *MCF* and $\Delta\varepsilon_{r,max}$ parameters were not expected to vary significantly as a function of hematocrit, since the active components of clotting (i.e., coagulation factors, platelets, and fibrinogen) were present at normal quantities in all samples. Spiezia et al. observed a similar trend in *MCF* with respect to hematocrit and characterized the phenomenon as an artifact of ROTEM as a fixed-volume viscoelastometric assay [12]. Specifically, at fixed volume, when the hematocrit decreases, the difference in volume is replaced by plasma. Since the fibrinogen concentration in plasma is constant, this results in an increased total amount of fibrinogen, which can polymerize into fibrin. As a viscoelastometric assay, ROTEM specifically probes the strength of the interaction between fibrin and platelets, so the *MCF* parameter can be sensitive to the increased quantity of fibrin. While ClotChip is also a fixed-volume assay, it is not a viscoelastometric assay and is thus potentially less susceptible to this phenomenon. In cases of diminished hematocrits, the ClotChip $\Delta\varepsilon_{r,max}$ parameter may in fact provide a better metric of clot firmness than the ROTEM *MCF* parameter.

Furthermore, both ROTEM *CT* and ClotChip T_{peak} parameters also increased with decreasing hematocrits down to ~10%. This may be due to the diminished surface area of phospholipid membranes available for facilitating the generation of thrombin via coagulation factors.

In comparing the ClotChip T_{peak} and ROTEM *CT* parameters, a relatively strong, statistically significant, and positive correlation was observed between the two parameters as shown in Fig. 4A.

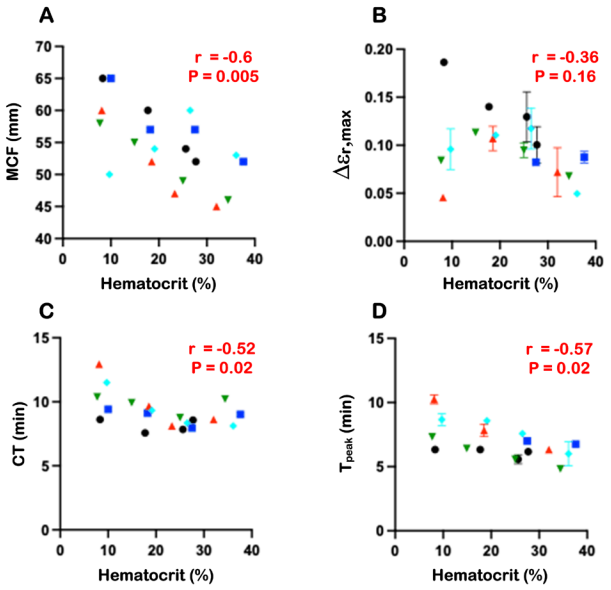

Fig. 3. Variation of ClotChip T_{peak} and $\Delta\varepsilon_{r,max}$ parameters as well as ROTEM *CT* and *MCF* parameters with hematocrit. The rWB samples were created from whole blood collected from five healthy donors, each indicated by a different colored marker. When shown, error bars are presented as mean ± standard deviation (SD) of duplicate measurements.

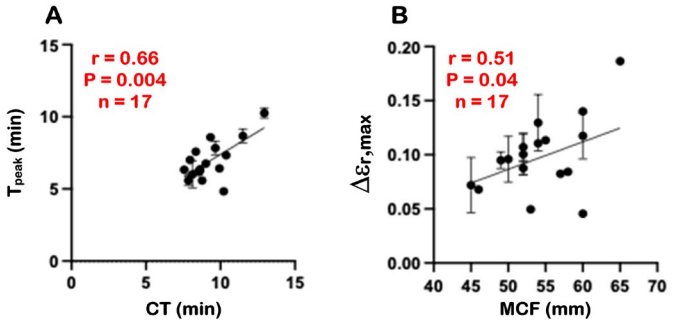

Fig. 4. The ClotChip T_{peak} and $\Delta\varepsilon_{r,max}$ parameters exhibited a relatively strong, statistically significant, and positive correlation with the ROTEM *CT* and *MCF* parameters, respectively. When shown, error bars are presented as mean ± SD of duplicate measurements.

The correlation between the ClotChip $\Delta\varepsilon_{r,max}$ and ROTEM *MCF* parameters was slightly less strong, but still significant (see Fig. 4B.) Taken together, these data indicate that the ClotChip assay is a viable dielectric blood coagulometer for hematocrits ranging from ~10–40%.

IV. Conclusion

In this work, we validated the utility of ClotChip as a dielectric blood coagulometer for assessment of hemostasis in cases involving diminished hematocrits. We established the presence of a characteristic dispersion region arising from the interfacial polarization of RBC membranes at hematocrits as low as ~10%. Moreover, we showed that the ClotChip metrics of clotting time and clot firmness correlated well with those measured by ROTEM for rWB samples with hematocrits in the range of ~10–40%.

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