Dielectric profile of blood clots to inform ischemic stroke treatments

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Adam Santorelli, *Member*, *IEEE*, Seán Fitzgerald, Andrew Douglas, Karen Doyle, and Martin O'Halloran

***Abstract*— Platelet and fibrin-rich blood clots can respond differently to red blood cell rich clots during ischemic stroke treatment, which includes thrombolysis and mechanical thrombectomy. Currently, there is no accurate way to identify the type of clot in advance of treatment. If the type of blood clot can be identified, the optimum clot removal process can be chosen and patient outcomes can be improved. In this paper we fabricate physiologically relevant blood clot analogues from human blood, that cover a range of red blood cell, fibrin, and platelet concentrations. We characterize the dielectric profile of these formed clots using an open-ended coaxial probe method across a wide frequency range. After the dielectric measurements are completed, histology on each blood clot is performed to determine the concentration of red blood cells present. In total, 32 unique blood clots were measured.**

With this completed analysis, we investigate the correlation between the dielectric properties across this frequency range and the red blood cell count of the formed blood clots. Furthermore, we develop a model to predict whether an unknown blood clot can be categorized as red blood cell rich or platelet and fibrin-rich based solely on the measured dielectric properties.

Clinical Relevance— **Using the dielectric profile of a clot, we can predict whether a clot is platelet and fibrin-rich or red blood cell rich, allowing clinicians to more easily determine treatment methods during an intervention for ischemic stroke.**

I. Introduction

Recent studies have suggested that clot composition is a main influence on the success of aspiration and stent retriever devices in mechanical thrombectomy. The physical interactions between the device used to retrieve the clot and the clot itself are highly dependent on the clot composition [1] – [4]. Identifying the composition of the clot, prior to treatment, could significantly impact treatment strategies and improve patient outcomes [4]. Additionally, it is believed that whether the clot is red blood cell (RBC)-rich or fibrin and platelet-rich (FP-rich) may play a particularly important role [5].

The dielectric properties of tissue, namely the relative permittivity, ε_r, and the imaginary component, ε'', define how electromagnetic (EM) fields and waves will interact with the tissues. A strong correlation between the dielectric properties of blood and its composition (primarily RBCs, white blood cells) has been shown in [6] – [9]. These recent publications suggest that using the dielectric profile of a blood clot may be used to help identify the composition of the clot. A measurement of the dielectric properties of a tissue can be carried out using compact and small-form technology, allowing for in-place, non-destructive measurements [10]. These benefits underscore the potential for clinicians to use dielectric measurements as a means to determine clot composition to better inform their treatment planning of stroke patients.

More recently, the idea to use the change in the electrical impedance to characterize and evaluate the formation of a clot was studied in [11]. In this study, researchers studied the changes in electrical impedance over the clot formation process using porcine blood. They noted different stages of the clot formation correlated to changes in the electrical impedance; furthermore, these stages of clot formation could also be seen to be related to the different proportions of fibrin and red blood cells. However, this work is based on relative changes in electrical impedance to identify the stage of thrombus formation, and as such can not provide insight into RBC and FP concentrations of the formed clot.

In this paper, we present a process to fabricate physiologically relevant blood clot analogues, fabricated from human blood, that span a range of RBC and FP concentrations. Using the open-ended coaxial probe method, we obtain a dielectric profile for each of these unique clots, and finally histological analysis is carried out to obtain the exact composition of each sample. Finally, we correlate the relationship between the dielectric properties and the RBC concentration of the clot. We demonstrate, for the first time that the dielectric profile of a given clot analogue may be used to predict whether a specific clot is RBC-rich or FP-rich, ultimately informing physicians on the best potential intervention for clot removal.

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II. Data Collection

A. Blood Clot Formation

Following approval from National University of Ireland Galway Research Ethics Committee, human whole blood was obtained from the Irish Blood Transfusion Service. The whole blood was centrifuged at 1,200 RPM for 20 minutes at 20°C to separate it into its constituents. Plasma was removed by pipetting and the remaining Red Blood Cells and Buffy Coat were mixed together by inverting. The plasma and RBC/Buffy-coat were then recombined in controlled ratios to form a range of different clot analogues from RBC-rich to FP-rich. Clotting was initiated by adding a 5% calcium chloride ($CaCl_2$) solution in a 1:9 ratio ($CaCl_2$ solution:blood mixture) in order to reverse the action of the anticoagulant. The blood clot mixtures were quickly loaded into plastic tubing and spun on a modified chandler loop system at 20 RPM for 1 hour until clots had formed. A photograph of two different blood clots, one a RBC-rich blood clot and the other a FP-rich blood clot is shown below in Fig. 1.

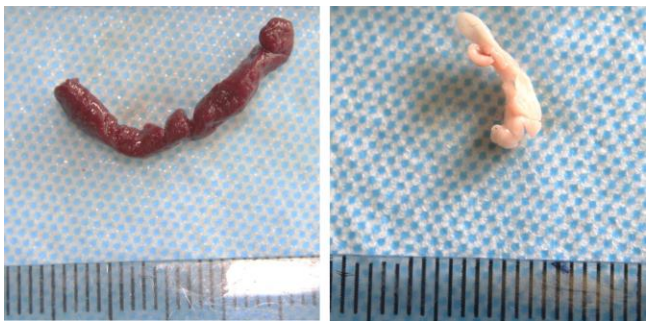

Figure 1. An example of two of the formed clots, comparing the shape and color of a RBC-rich clot (left) and a fibrin/platelet-rich clot (right).

B. Dielectric Property Measurements

Permittivity measurements were performed using the open-ended coaxial probe technique. Data was collected using the Keysight slim form probe (2mm diameter coaxial probe) [12] connected to the Keysight E8362B Network Analyzer (NA). The probe was connected directly, minimizing any measurement uncertainty associated with cable movement and repositioning [13], [14]. Data was collected over the 500 MHz – 20 GHz frequency range, with 301 log-spaced data points recorded [12]. Three measurements were performed for each clot sample.

A standard three-load one-port calibration (Air/Short/Deionized water) was used. A validation measurement was performed after each calibration using 0.1 M NaCl solution. Following the procedure outlined in [8] and [13], a combined uncertainty of less than 4% was calculated for ε_r and ε''. The combination of a vertical stand, and *x-y* positioning tables was used to move the blood clot sample into contact with the dielectric probe. All measurements were performed with the samples at room temperature, the temperature of the samples ranged from 24.1 – 25.1 ºC). An image of this experimental setup is shown in Fig. 2.

C. Histological Analysis

Gross photographs of each clot analogue were taken immediately after the dielectric measurements. All clot analogues were then immediately fixed in 10% phosphate-buffered formalin for a minimum of 24 hours, processed using a standard tissue processing protocol and embedded in paraffin (FFPE). The FFPE clot material was cut into 3µm sections and two sections from each analogue were mounted on each slide. A representative slide from each clot was stained with Hematoxylin and Eosin (H&E) and Martius Scarlett Blue (MSB). An MSB stained slide from each clot analogue was scanned at 20x magnification using an Olympus VS120 slide scanner. Histologic quantification was performed on the digital slide scan file (.vsi) using Orbit Image Analysis Software (www.orbit.bio). The relative proportions of RBCs, fibrin, and platelets within each clot analogue were quantified.

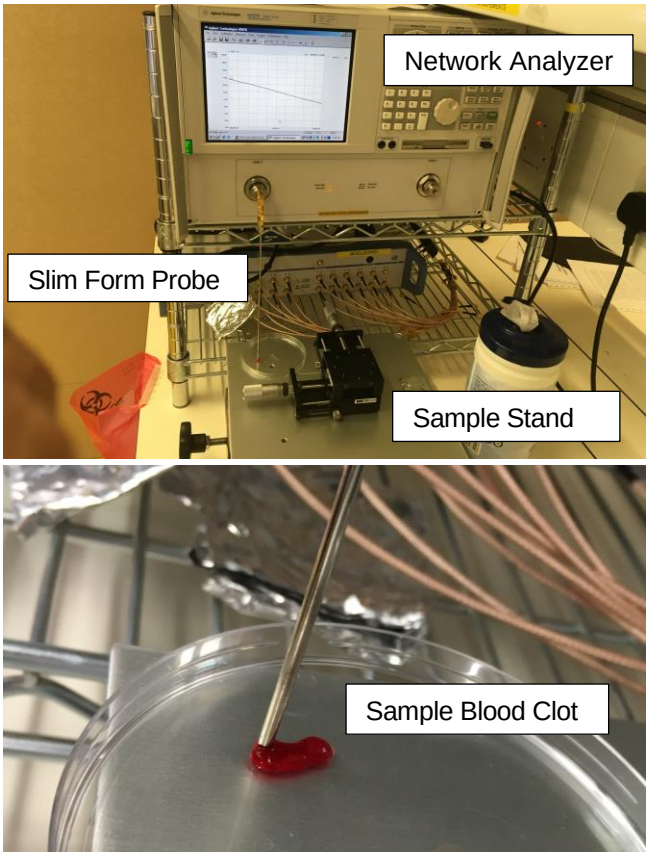

Figure 2. A photograph of the measurement setup and equipment needed for the dielectric measurements (top) and a zoomed-in image of the sample blood clot in contact with the dielectric probe (bottom).

III. Results

The range in the relative permittivity of the measured blood clot samples across the 32 unique blood clots is shown in Fig. 3. The curves in the plot are color coded based on RBC and FP concentration (pink indicates low RBC concentration). These measurements indicate that there is a wide range in the dielectric properties of blood clots over the 0.5 – 20 GHz range, agreeing with the measurements of

blood samples in [6] – [8]. At 915 MHz, an approved ISM band and thus a popular choice for medical device development, the relative permittivity varies between 51.69 and 65.79 and ε'' varies between 19.57 and 28.98.

The development of an accurate prediction model is reliant on precise knowledge of the composition of the clot. The results of the histological analysis into the blood clot composition is shown in Fig. 4. These results confirm our ability to create blood clots that cover the entire range of clot compositions, from RBC-rich to FP-rich.

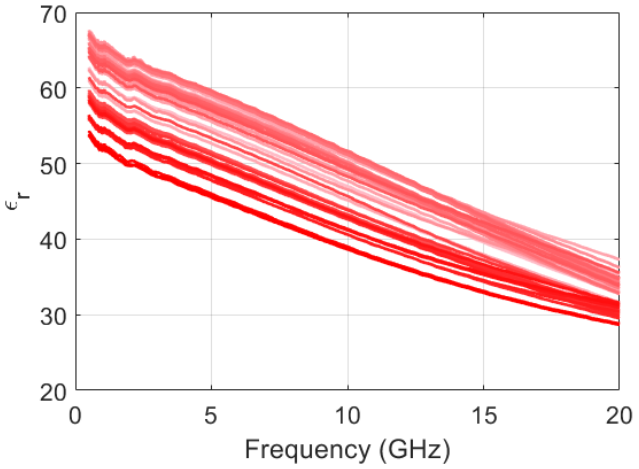

Figure 3. Range of the measured permittivity across the recorded frequency band for all 32 distinct blood clots, this data includes the multiple measurements performed on each clot. The color is correlated with the RBC concentration (pink indicates low RBC and red indicates a RBC-rich clot).

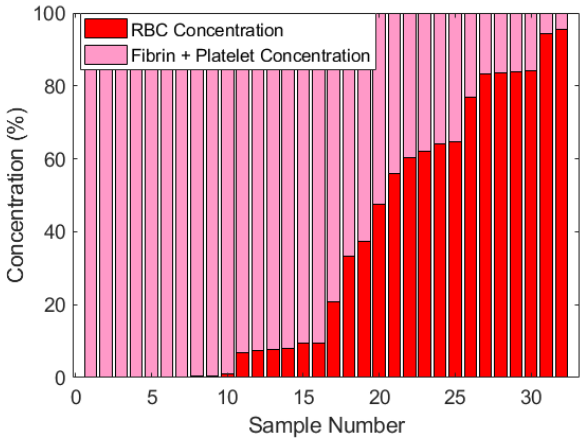

Figure 4. Results of the histological analysis of each blood clot, indicating the concentration of RBCs in comparison to the concentration of fibrin and platelets.

An investigation between the correlation of the dielectric properties and the RBC concentration was carried out at 915 MHz, due to its popularity and ease of use for medical device development. A strong correlation between the dielectric properties and the RBC concentration of the clot implies that a medical device in this ISM frequency band would offer promise for blood clot identification. An exponential regression, defined in equation 1 below

$$y \sim 3.3 \times 10^{8} \times e^{-0.27x} \quad (1),$$

where y is the RBC concentration in percent and x is the relative permittivity, can be used to represent the relationship between RBC concentration of a blood clot and the permittivity. This relationship, with an R^2 value of 0.78, is also plotted in Fig. 5. A similar relationship also exists for the imaginary component of permittivity.

This strong correlation between the clot composition and the dielectric properties was a promising sign into the development of a predictive model. We used the real and imaginary component of the permittivity at 915 MHz as the two features in a prediction model. An example of the space created by these two features is shown in Fig. 6, with each class defined as an RBC-rich or FP-rich clot. The cut-off for a FP-rich clot was chosen to be a maximum of 49% RBC concentration [5]. The RBC-rich clot class is shown in red, with the FP-rich clot class shown in blue, respectively, in Fig. 6. From this subspace plot, it is clear that even two features at a single frequency point can be used to separate the data between RBC-rich and FP-rich clots. For example, even an intuitive thresholding prediction of $\varepsilon_r > 59$ and $\varepsilon'' > 23$, seems to be a promising indicator to identify the FP-rich clots. Additionally, as an illustrative example, a Gaussian-based kernel, support vector machines (SVM) classifier was trained with the above data and obtained a sensitivity and specificity of 95% and 100%, respectively, in cross-validation training. These results are a promising indication that the dielectric profile can be used to differentiate between RBC-rich and FP-rich clots.

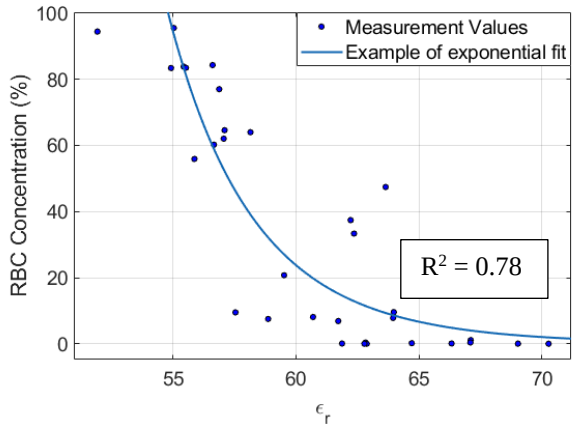

Figure 5. An example of an exponential fit modelling the changes the RBC concentration of a blood clot with changes of the real component of permittivity.

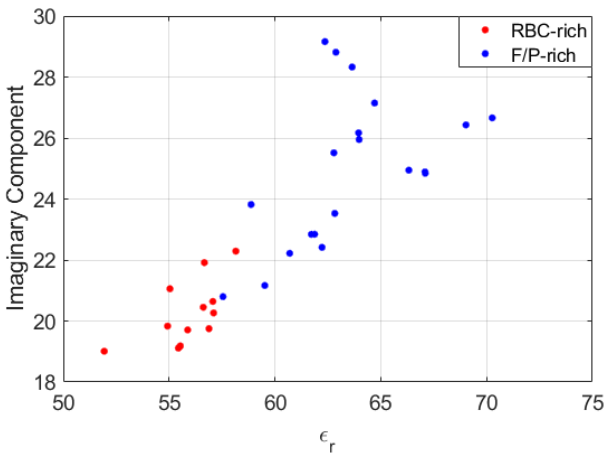

Figure 6. An example of the dielectric profile created by using the two features, real and imaginary component of permittivity, to map out FP-rich or RBC-rich clots. The RBC-rich clots are grouped together in the lower-left hand corner (low real and imaginary component).

IV. Conclusion

This paper demonstrates that physiologically relevant human blood clot analogues spanning a range of red blood cell and fibrin and platelet concentrations can be characterized by their dielectric profile. A strong correlation between the dielectric properties and the blood clot composition was found. This correlation exists at the 915 MHz frequency, an ISM band that is popular for medical device development. Additionally, we demonstrate the possibility of using the dielectric properties, the real and imaginary components, as features in a predictive model to distinguish from RBC-rich and FP-rich clots. While this paper is a preliminary investigation into such a predictive model, with a limited number of unique blood clots (32 distinct clot concentrations were investigated) an initial sensitivity and specificity of 88% was obtained. These results indicate the promise in the development of a medical device that can be used *in situ* to determine the composition of a clot, based on its dielectric profile, to help inform clinicians on treatment planning for stroke patients, and ultimately improve patient outcomes.

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