RESEARCH PAPER

Microwave dielectrometry as a tool for the characterization of blood cell membrane activity for *in vitro* diagnostics

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This paper deals with the concept of microliter sensing of human blood samples by waveguide dielectrometry at microwave frequency (39.5 GHz). The methods, research protocol, and basic results derived from the permittivity measurements are presented. Here we summarize the results of our 5-year research on developing a multidisciplinary approach to the characterization of erythrocytes dielectric response, in parallel with biochemical studies of their receptor-membrane activity, within the scope of a case–control study for in vitro diagnostics of acute and chronic neurological conditions.

I. INTRODUCTION

The advances in microwave technologies have contributed to their widespread usage from industry and engineering to healthcare. Being contactless, label-free, and reasonably inexpensive, microwave-based techniques are particularly attractive for biomedical applications. An essential advantage is non-destructive monitoring in real time being most important for living biological systems. Permittivity measurements, commonly over a relatively wide range of frequencies, provide valuable information on structural and functional properties of various biological objects. Thus, the existing techniques and associated metrology permit to accurately discriminate pathological conditions [1, 2] and to observe different biological processes [3, 4]. Still, there is no optimal or universal tool for that. Evidently, any implementation as well as a sensor design should always match the specific requirements of a study and sample properties. So the development of new analyzing instrumentation and their real-world application are highly challenging. It may concern certain difficulties in usability during routine manipulations by medical staff, accurate calibration of sensors to be used in actual clinical

practice, and other requirements on a test such as desired accuracy and sensitivity that are strongly influenced by the effect size, and data reproducibility.

Another big challenge when dealing with data derived from biomedical studies is that they are commonly non-normally distributed [5, 6] and may contain lots of missing values. Actually, it depends on a specific character of the parameters being studied. For instance, some values may contain outliers, or may be skewed due to the bias and confounding effects by multiple factors (age, gender, individual differences). For such non-normal variables, or small samples with large deviations from normality, standard statistical analysis may lead to incorrect inferences, so one should consider alternative approaches: data transformations, non-parametric tests, or statistical bootstrapping [5].

The main focus of this work is the applicability of dielectric blood sensing in biomedical research and, most significantly, acquisition of clinically meaningful data derived from the dielectric measurements.

II. CONCEPT OF DIELECTRIC BLOOD SENSING

Specifically for blood measurements, the extensive results on dielectric properties of whole blood and its components are widely covered in literature. However, most results are from spectroscopy rather than at a single frequency. The approach to sensing we describe here supposes the permittivity measurements of red blood cells (RBCs) at a fixed frequency of 39.5 GHz, which corresponds to the γ-dispersion domain. This domain is attributed to a relaxation of free water molecules and occupies a large portion of the microwave range,

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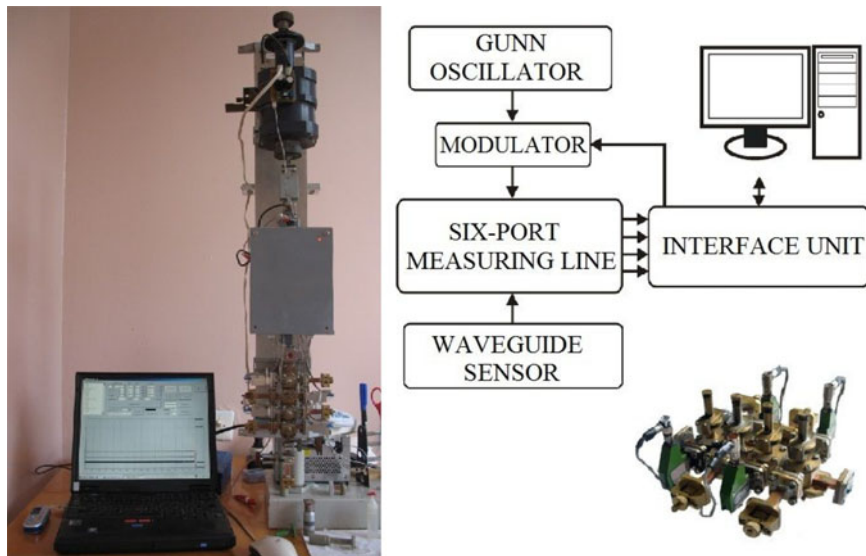Email: arkhykate@gmail.com

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Fig. 1. Measuring setup based on a six-port measuring line and waveguide sensor.

but the changes in dielectric properties of biological objects are recognized throughout whole spectrum [7]. Thus, we can implement our measurements at any single frequency within the 10–60 GHz range. In this particular case, the selection of such a frequency was influenced by: (1) waveguide size (5.2 × 2.6 mm) used in sensor design for measuring very small sample volumes (microliter); (2) operating only on the fundamental mode; and (3) a wavelength in waveguide, which should be in consistent with manufacturing tolerances of sensor elements. The main point is that we aim to detect clinically significant effect of $\Delta\varepsilon^*$ rather than a frequency band where it occurs.

The idea to apply millimeter-wave dielectrometry specifically for clinical purposes goes back a long way, from the late 1980s onwards, when a series of control experiments revealed correlation between the dielectric behavior of different biological objects and physiological conditions [8]. Cumulative results and numerous observations on the dielectric response of RBCs to pharmaceuticals (hormones and blockers) motivated us to develop the technique for *in vitro* monitoring of adrenergic-receptor activity, which plays a crucial role in the pathogenesis of cardiovascular diseases. The interdisciplinary approach we use involves the analysis of $\Delta\varepsilon^*$ of RBCs induced by the effect of β-blockers (BB) – the drugs commonly used in treatment of certain cardiovascular disorders – using the combination of theoretical and experimental techniques.

A) Single-frequency waveguide dielectrometry

The technique is based on the measurement of the complex reflection coefficient R^* from a short-circuited multilayer structure (waveguide sensor) containing a sample by means of the six-port reflectometer (see Fig. 1) under the single-frequency mode ($f = 39.5$ GHz). Signal power in the waveguide transmission line does not exceed 10 mW; measurement time is 4 s per sample. A more detailed discussion of the measurement setup can be found in [9].

The sensor is a short-circuited segment of rectangular waveguide (5.2 × 2.6 mm²), which holds up to 5–7 µl of sample with thickness of about 100 µm (Fig. 2). A distinguishing feature of the sensor is its measuring sensitivity improvement at the operating frequency by additional dielectric inserts of different shapes (Figs 2(c) and 2(d)) that has been realized specifically for the repeated microliter measurements of blood samples [10].

Such sensor modifications have been studied with use of the two-dimensional finite-difference time-domain technique [11] and the software package written in Python both for optimization and calibration of the measuring structure. We used pure water and ethanol/water mixtures with mole fractions of 0.04 and 0.08 as reference liquids [12] both for simulation and measurements. Standard deviation of 30 measurements of each reference liquids is ∼1.2% in average and ∼2% at a maximum. Our results showed that the required accuracy of the calibration, which should be at least 2.5%, can be achieved by means of the proposed procedure. Further details concerning the calibration procedure can be found in [13].

B) Parallel method

To evaluate receptor-membrane reaction and to validate the results of dielectric study, we have opted for the osmotic
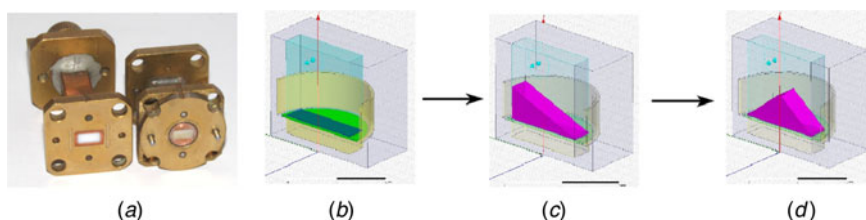

Fig. 2. Waveguide measuring sensor (a) and its simulation models: basic structure (b) and modified by dielectric inserts (c, d).
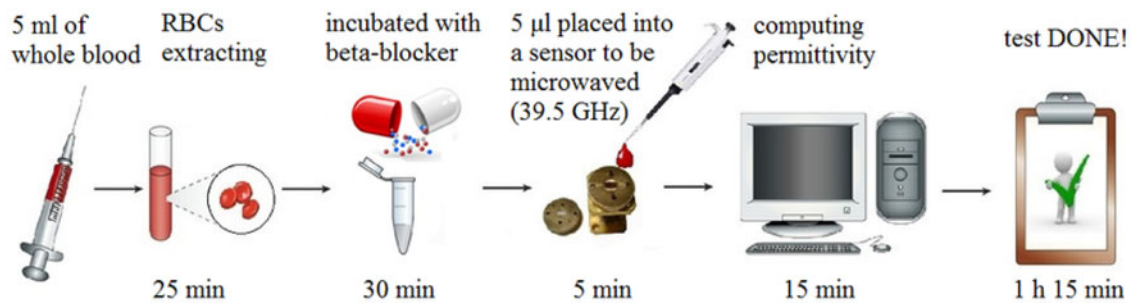
Fig. 3. Infographics of the research protocol.

fragility test. It refers to the degree of susceptibility to hemolysis (i.e., a disintegration of RBCs with the release of hemoglobin into the intercellular medium) that occurs when RBCs are subjected to osmotic stress by being placed in a hypotonic solution. The method we used exploits a principle of hemolysis inhibition in the presence of BB propranolol, which binds to β-adrenergic receptors (β-ARs) on red-cell membranes and thereby reduces damage degree [14]. The β-ARs activity is numerically expressed by β-adrenergic reactivity of membranes (ARM) index; according to the guideline in [14], about 93% of healthy individuals have reference values in the range 2–20 units. The index values and receptors activity have reciprocal correspondence: β-ARM index will be numerically increased as the osmotic resistance is decreased, and vice versa. This method is commonly used for the diagnosis of hyper-adrenergic states, prognosis of cardiovascular diseases, and assessment of individual sensitivity to BB in therapy.

C) Research protocol

The research protocol is based on tracing drug-induced changes in RBC permittivity measured before and after the effect of BB. Measurements were performed using RBC samples (hematocrit = 0.85) extracted from heparinized venous blood (5 ml) pretherapy collected from patients with neurological disorders. More details about the sample preparation can be found in [15]. The samples were subdivided into two groups (A, from $n = 31$ with chronic encephalopathy; and B, from $n = 29$ with acute stroke). The comparison group (control) comprised healthy individuals ($n = 27$, aged 55.2 ± 9.4) without any medical intervention whose indicators were baseline. To characterize β-ARs activity, the RBC samples were incubated with BB propranolol ($C = 7.5$ g/l) for 30 min at room temperature ($25°C$). The measurements of permittivity are carried out immediately after the incubation. All manipulations with blood were conducted during 3 h after its sampling (Fig. 3).

Data handling and statistical analysis were performed using free software based on programming language R [16]. It also allowed us to create a multiparameter database, which in addition to permittivity measurements contains other indicators: patient demographics, clinical data, sample temperature, the results of repeated measurements when necessary, etc., thus enabling us to consider all important information during analysis.

III. EXPERIMENTAL RESULTS

The results of the study are presented as box plots (Figs 4 and 5). They include summarized data of permittivity measurements

of RBC samples as part of the case–control study (i.e., comparison of dielectric response in health and diseased patient samples) and information on the statistical inferences (P-values). We opted for non-parametric tests since some raw data were skewed and did not follow a normal distribution. Hence, comparing medians (ranked data) rather than mean values is preferred because the median is not affected by extreme values. Within-group differences (Fig. 4) were analyzed using the Wilcoxon sign-rank test, whereas between-group differences (Fig. 5) using the Wilcoxon rank-sum test (also known as the Mann–Whitney U test).

Fig. 4 shows the distribution of measured absolute values of RBC permittivity (ε' and ε'' separately) in all study groups, where their variability outside the upper and lower quartiles (whiskers), the medians and means (marked with a dot) may be observed. Here, we analyze the response of erythrocytes to the BB effect, i.e., the comparison of permittivity values of the samples of the same individual with and without the drug (with equivalent adding of physiological saline instead).

It should be also noted that since ε^* measurements have been conducted at different times, the correction for temperature of samples was made. Thus, we adjusted all absolute values to $25°C$ (average value); the procedure is partially described in [17].

As illustrated (in Fig. 4), there are statistically significant differences in the permittivity of RBCs of the control group and those patients with mild form of neurological disorder (group A), whereas in those with an acute stroke (group B), the change in ε' to the drug is statistically non-significant. The same effect can be observed in imaginary part of the permittivity.

However, the observable effects and the main idea of the developed test are more clearly illustrated in Fig. 5. It demonstrates how exactly the dielectric response ($\Delta\varepsilon^*$) of red cells to BB effect is distributed in health and disease.

As can be seen from the graph (Fig. 5), drug-induced $\Delta\varepsilon^*$ depends on physiological condition of blood donors and can be detected with use of microwave dielectrometry. This particular finding indicates a high potential of the method for diagnostic purposes.

The data derived from the dielectric study are in good agreement with the results of the parallel test (β-ARM indices) represented in Table 1.

The degree of β-AR activity of erythrocytes is essentially reduced in the diseased groups (A and B) compared with the control; this is apparent from the indices, which exceed the reference ones (2–20 units), which, in turn, correspond to low-level receptors activity caused by a disease [14].
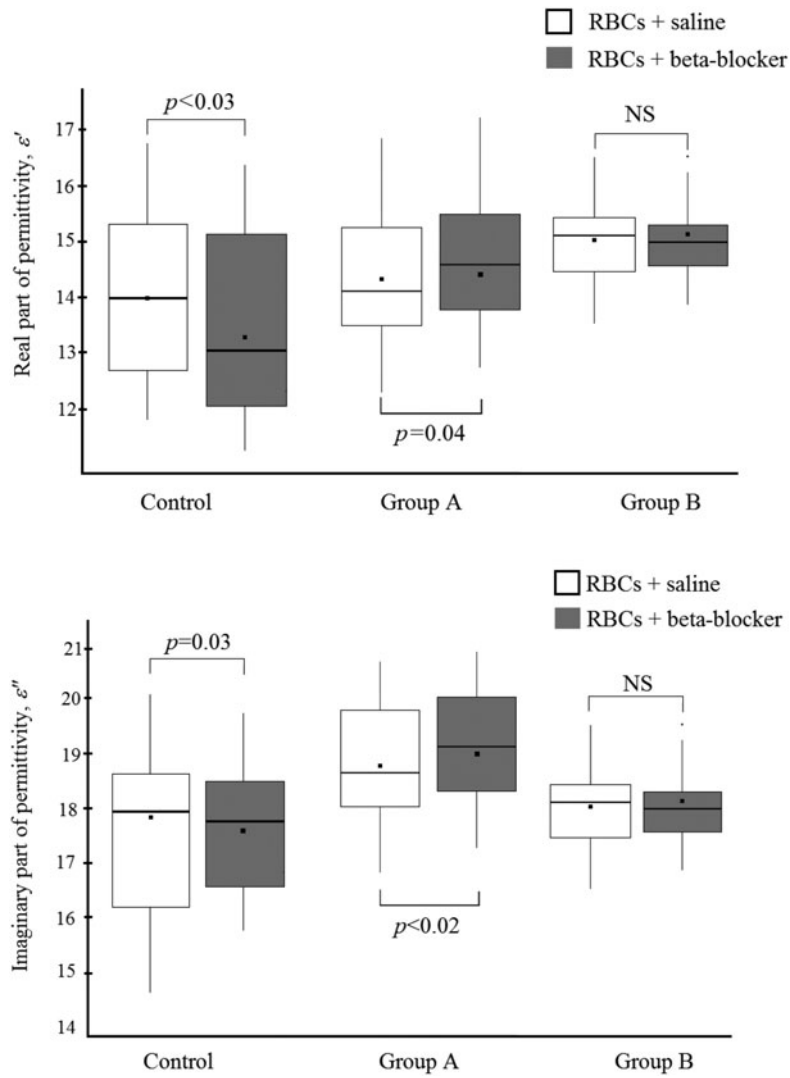
Fig. 4. Complex permittivity of human erythrocytes ($f = 39.5$ GHz, $T = 25°$C) in the control and the diseased groups (A and B) before and after the drug effect.
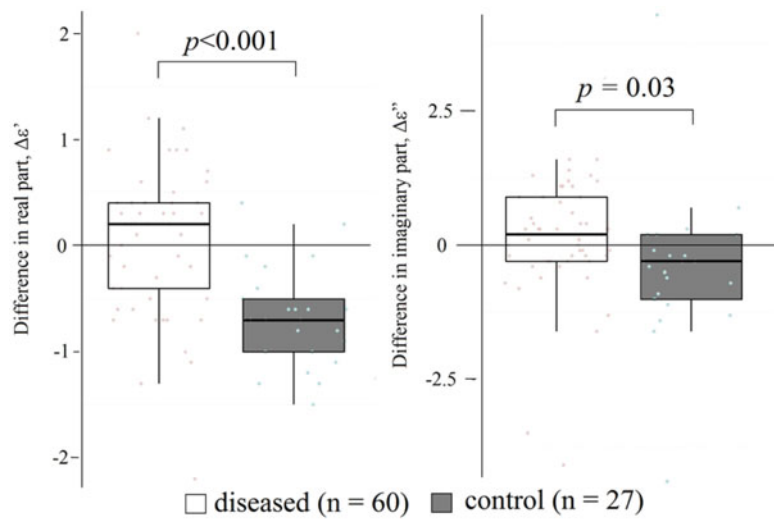

Fig. 5. Comparison of the RBC dielectric response ($\Delta\varepsilon^*$) to the BB in health and disease.

Table 1. Measured values and statistical inferences.

Group	β-ARM index, mean value	$\Delta\varepsilon^*$, median				Spearman's correlation coefficient (β-ARM/$\Delta\varepsilon^*$)
		$\Delta\varepsilon'$	P-value	$\Delta\varepsilon''$	P-value	
Control, $n = 27$	16.1 ± 4.6	-0.71	$<0.03^*$	-0.30	0.03^*	$r = +0.3$, $p = 0.02$
A, $n = 31$	34.6 ± 5.4	0.35	0.04^*	0.30	$<0.02^*$	
B, $n = 29$	42.2 ± 6.1	0.0	NS**	-0.1	NS**	

*P-value < 0.05 is statistically significant, **NS denotes statistically non-significant difference.

Thus, the statistically significant ($r = +0.3$, $p = 0.02$, the Spearman rank) correlation between the magnitude and sign of dielectric response and β-ARM indices is observed. A lowering of the permittivity is observed only in healthy individuals, which confirms the effect previously observed in [15] with the lesser sample size.

IV. CONCLUSION

The applicability of dielectric techniques in the biomedical area is highly dependent on study design, specific properties of samples, and other technical and methodological aspects that should be considered. The described multidisciplinary approach to the characterization of cellular membrane activity is based on blood cell permittivity measurements in conjunction with the parallel biochemical testing and is intended to supplement current cellular analysis used for healthcare. We imply that the approach may be helpful for specialists dealing with dielectric measurements and the developers of clinically oriented instrumentation based on microwave dielectrometry.

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