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Electrical impedance assays of blood cells

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ABSTRACT

In this review, the capability of electrical impedance spectroscopy analysis of blood cells, especially for red blood cells is presented, highlighting its large area of related biomedical relevance. The method is briefly introduced and basic theoretical aspects are discussed by considering both phenomenological (e.g. equivalent circuit) and microscopic approaches. The latter include a comparative analysis of the relevance of considering real shape (consistent with microscopic observations) versus spheroidal approximations (prolate and oblate spheroids) with the same surface and volume concentration. We show that while ellipsoidal approximation is fairly good for randomly oriented cells, it is quite poor whenever oriented cells are measured. The voluminous literature on the electrical analysis of blood cells is reviewed to stress the most promising biomedical applications of the method either per se or in combination with complementary e.g. (micro) fluidic approaches.

Keywords: electrical impedance spectroscopy, equivalent circuit, microscopic approach, prolate and oblate spheroids, red blood cell aggregation

Abbreviations: electrical impedance spectroscopy (EIS), red blood cells (RBCs), white blood cells (WBCs), red blood cell aggregation (RBCa), sample under test (SUT), geometric factor of the SUT(GESUT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), cardiovascular diseases (CVDs), prothrombin time (PT), partial thromboplastin time (PTT), and thrombin clotting time (TCT), electric impedance microflow cytometry (EIMC), sickle cell disease (SCD), acquired immunodeficiency syndrome (AIDS).

INTRODUCTION

Blood is a complex heterogeneous functional fluid that transports physiological gases as well as nutrients and metabolites throughout the body. It is primarily composed of red blood cells (RBCs), normally accounting for about 45% of the total blood volume. The rest consists of plasma, and other components representing less than 1% of the blood volume (white blood cells, platelets, etc.). Clearly, quantitative assessment of blood properties is highly relevant in medicine and biology. For diagnostic purposes, a plethora of investigation methods addressing haematological and biochemical parameters of blood as well as the quest for various disease biomarkers have been advanced. These methods involve molecular, optical, (micro) fluidics or electrically (including electrochemically) assays[1, 2]. Despite the wide range of available technologies, when it comes to the blood of wild animals it is still difficult to find an easy and affordable solution for multiple species. The optical microscope was the primary method for cell classification and counting in human beings and animals. However, microscopy is still highly dependent on skilled laboratory personnel[3].

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The aim of this review is to highlight and summarize currently available electrically based methods to analyze blood components in relation to relevant biomedical aspects, as well as to emphasize several related (theoretical) limitations.

ELECTRICAL IMPEDANCE SPECTROSCOPY

Electrical impedance spectroscopy (EIS) is a powerful analytical method devised to characterize the non-homogeneities within a sample under test (SUT). EIS is carried out by applying a sine-wave voltage signal with frequency v, $V(t,v)$ and measuring the induced current, $I(t,v)$. The electrical impedance is the complex parameter defined as the voltage versus current ratio, as shown in eq. 1:

$$V^*(T,v)=V_0(v)\cdot\exp(2\pi vt);\ I^*(t,v)=I_0(v)\cdot\exp(I\cdot(2\pi vt+\phi))$$
$$Z^*_{SUT}(v)=\frac{V^*(t,v)}{I^*(t,v)}=\frac{V_0(v)}{I_0(v)}\exp(-I\phi) \quad (1)$$
$$Z^*_{SUT}(v)=\mathrm{Re}(Z^*_{SUT}(v))+I\,\mathrm{Im}(Z^*_{SUT}(v));\ |Z^*_{SUT}(v)|=\frac{V_0(v)}{I_0(v)}$$

The complex dielectric (permittivity) parameter is related to the complex impedance via the geometric factor of the SUT, GF_{SUT}.

$$\varepsilon^*_{SUT}(v)=\frac{GF_{SUT}}{2\pi v\cdot Z^*_{SUT}(v)};\ \varepsilon^*_{SUT}(v)=\varepsilon_{SUT}(v)+\frac{\sigma_{SUT}}{I\,2\pi v\cdot\varepsilon_0} \quad (2)$$

ε_{SUT} denotes the real part of SUT permittivity, σ_{SUT} stands for SUT conductivity and ε_0 represents vacuum permittivity.

Aiming for label free blood analysis, electrical impedance assays have been extensively approached since the end of the 19th century, when Stewart[4,5] discovered that the electrical conductance of blood decreases with increasing the erythrocyte concentration. The pioneering studies of Höber, Fricke and Cole[6–11] revealed the equivalent circuit in RBCs, consisting of Rp (plasma resistance) in parallel with a series circuit given by Ri (resistance of cell interior) and Cm (membrane capacitance), from which the experimentally related electrical parameters were derived ***(Fig. 1)***.

RBC count plays an important role in animal diagnosis. Automatic counters that produce more specific results and thus apply less subjective criteria were proposed. A revolutionary method for counting RBC based on the electrical impedance variation of the blood fluid and its suspended particles, when passing through a sensing aperture in a capillary was introduced by Coulter[12].

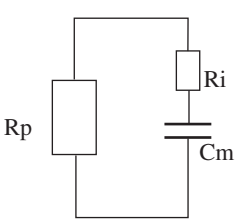

Fig. 1 **Equivalent circuit of the red blood cells.** It consists of Rp (plasma resistance) in parallel with a series circuit given by Ri (resistance of cell interior) and Cm (membrane capacitance).

Nurtured by both theoretical (via microscopic or phenomenological approaches) and experimental (including instrumentation) advancements, EIS has been extensively developed during the last century to become one of the first hand tools capable of non-invasively monitoring the electrical properties of cells and tissues[13–18].

THEORETICAL ASPECTS FOR DERIVING RED BLOOD CELL PARAMETERS FROM ELECTRICAL IMPEDANCE MEASUREMENTS

To derive quantitative data from electrical impedance measurements on cells and tissues, one has to consider either phenomenological approaches or microscopic models. While the phenomenological models are based on equivalent circuits comprising passive elements e.g. resistors and capacitors and/or mathematical expressions without a clear, intuitive physical representation[15,19], the microscopic approaches provide potential and charge spatial distribution via analytical expressions derived using Maxwell equations and structural information from various investigation systems and approaches e.g. biological, optical microscopy, etc. Aiming to simplify analytical expressions, the rather complex shape of biological cells has been traditionally simplified to spheroidal if not to spherical ones. In the following we highlight the errors that may accompany spheroidal assessments instead of the actual shape of biological cells or tissues with emphasis on RBCs. While the detailed theory is presented in our previous papers[20–23], here we discuss the shape effect on the dielectric (impedimetric) behavior of a suspension of RBCs. The whole range of RBC shapes (presenting axial symmetry) are simulated by considering the following expression:

$$R[\theta]=\sqrt{-A\cdot\cos(2\theta)+\sqrt{C^2-(A\sin(2\theta))^2}};A=\frac{(d^2-t^2)}{8};C=\frac{(d^2+t^2)}{8}) \quad (3)$$

Where θ denotes the polar angle, d represents the RBC diameter (i.e. the distance between the lower and the upper points in the RBC contours, represented in ***Fig. 2***) and t stands for the RBC thickness i.e. the distance between the points at the intersection of the RBC contours and the symmetry axes (the horizontal one) in ***Fig. 2***.

Fig. 2 reveals the RBC shapes according to eq. 3 and the coordinates provided in ***Table 1***.

Table 1 **Red blood cells parameters related to equation 3**

No.	Diameter (μm)	Least thickness (μm)
1	7	0.8
2	6	1.0
3	8	0.5

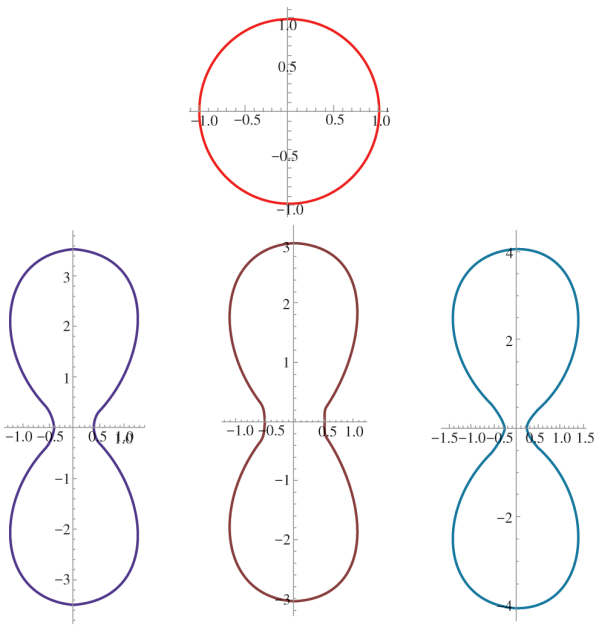

Fig. 2 **The shapes considered for the red blood cells and a spherical one (one to three from left to right, with parameters in *Table 1*).** The symmetry (rotation) axes is the horizontal one.

Considering a microscopic model[21], one can derive the complex permittivity of a RBC suspension as given by:

$$\varepsilon_{sut} = \varepsilon_{out} + p \cdot \frac{p \cdot \sum_j k_j \alpha_j}{1 - p/3 \cdot \sum_j k_j \alpha_j} \quad (4)$$

Where ε_{out} denotes the permittivity of the plasma, p represents the volume ratio of the cells (p = Vc/Total Volume) and α_j the polarizability corresponding to a specific orientation that has assigned the weight k_j (j=1÷3).

We have considered shelled particles (membrane thickness, mt=10^{-2} μm) with the following values for the electrical parameters: ε^*_{in}=70.1− I 0.2/($2\pi\nu\varepsilon_0$), ε^*_{shell}=12, ε^*_o=78− I 0.377/($2\pi\nu\varepsilon_0$) and p=0.5 Vshape/(4 Pi/3 Rsf^3); Rsf corresponds to the radius of the sphere with the same surface as the RBCs; the same surface and the same number of cells was assessed on all RBC shapes.

The dielectric behaviors of prolate and oblate spheroids (obtained by rotating an ellipse about its major axis, and minor axis respectively), having the same volume and surface as the actual red blood cell (with shapes described by eq.3) were computed and compared with the spectra of the real shapes. Whereas similar behavior (spectra) is derived when considering the suspension´s randomly oriented cells (k_j =1/3), significant differences between simulations based on actual versus spheroidal shapes are obtained for oriented cells, especially when the electric field is perpendicular to the cell rotation axes (symmetry), as indicated in ***Fig. 3***.

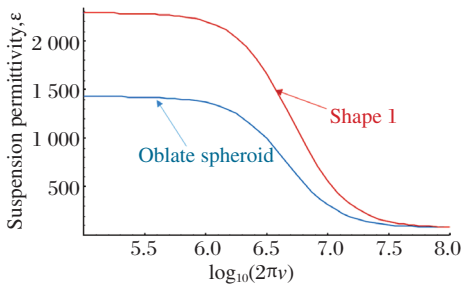

Fig. 3 **The permittivity spectra for shape 1 (defined in *Table 1*) and the corresponding oblate spheroid when the electric field is perpendicular to the cell rotation axes.** The electric field is perpendicular to the cell rotation/symmetry axes.

USE OF ELECTRICAL IMPEDANCE MEASUREMENTS TO ASSESS BLOOD PROPERTIES AND RELATED BIOMEDICAL/DIAGNOSIS RELEVANCE

RBCs possess a unique capacity for undergoing cellular deformation to navigate across various human microcirculation vessels, enabling them to pass through capillaries that are smaller than their diameter and to carry out their role as gas carriers between blood and tissues. Since there is growing evidence that red blood cell deformability is impaired in some pathological conditions, measurement of RBC deformability has been the focus of numerous studies over the past decades[24]. Nevertheless, reports on healthy and pathological RBCs are currently limited and, in many cases, are not expressed in terms of well-defined cell membrane parameters such as elasticity and viscosity. Hence, it is often difficult to integrate these results into the basic understanding of RBC behavior, as well as into clinical applications[25]. Blood analysis is currently performed using various technologies that frequently include electrical impedance measurements and flow cytometry.

An impedance measurement system provides accurate and fast analysis of constituent components of a (liquid) sample. While electrical impedance still has a firm position in determining the overall number and size of cells, flow-cytometry techniques have proven

their capability to differentiate white blood cells and identify abnormal cells[25]. Most automated blood cell counters assess blood parameters: hemoglobin content of RBCs, hematocrit, RBC count, mean corpuscular volume (MCV) of RBCs, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, mean platelet volume, and white blood cell(WBC) count. Quantitation of the RBCs, WBCs, and the platelets can be achieved by electrical impedance assay; following Coulter[12], this approach relies on the conductance change accompanying cells passing through a small aperture[26].

According to the World Health Organization, cardiovascular diseases (CVDs) account for about one third of total mortality all over the world. Patients with serious CVDs present microcirculatory disorders found to be strongly related with blood properties that are widely tested to diagnose the pathological or physiological disorders of CVDs[27]. Among the biophysical properties of blood, RBC aggregation is the major cause of the non-Newtonian flow properties of whole blood, and has been popularly utilized using electrical impedance spectroscopy to diagnose inflammatory diseases in clinic, since the level of aggregation rises enormously in association with diabetes, sepsis and myocardial ischemia[27–29].

RBC AGGREGATION (RBCA)

RBC aggregation (in the form of rouleaux) can be altered during pathophysiological processes. Aggregation affects the flow properties of blood, especially at low shear rates, and therefore has the potential to influence blood flow in the circulatory system. There are several classes of RBC aggregation. Linear RBC aggregates are called rouleaux. The number of RBCs per rouleau can vary and branching into two rouleaux can occur[30]. Reversible rouleaux formation is caused by plasma macromolecules; however, several RBCs might become actively adhesive in the presence of a blood clot (e.g. caused by the activation of platelets). Clot formation can be life-saving in the case of wound healing, but also a major cause of death in the case of a thrombus induced stroke. Moreover, there are several pathological cases such as malaria or sickle cell diseases where red blood cells are known to form large aggregates that hinder the flow of blood[30]. The factors determining RBC aggregation are both cellular (e.g. RBC shape, glycocalyx, oxidant stress) and extracellular e.g. flow condition, pH value, osmotic pressure, concentration of plasma proteins (in acute phase reactions). Both in coagulation and aggregation, fibrinogen plays a crucial role; without any macromolecules, e.g. RBCs in a simple salt solution, no aggregation occurs[30,31]. Whereas the electrical properties of plasma and blood cells provide fundamental insights into the health status of patients, including the detection and evaluation of thrombus formation, the potential of EIS is not fully exploited today[32, 33].

Routine coagulation tests are set to provide rapid information on the general (relatively non-specific) nature of an abnormality and direct the clinician to a diagnosis by analyzing distinct coagulation factors. The parameters normally used to screen for haemostatic defects are prothrombin time (PT), partial thromboplastin time (PTT), and thrombin clotting time (TCT). PT is also widely used to monitor the treatment of patients receiving oral anticoagulant therapy. The data associated with the onset of coagulation provided by electrical impedance assays are comparable to those derived from absorbance measurements. The amplitude of the impedance change correlates well with the fibrinogen concentration of the plasma[34]. Quantitative assessment of blood coagulation is essential to predict the risk of hemorrhage and thrombosis during cardiac surgical procedures. Electrical impedance based devices integrating high throughput microfluidics were developed to assess blood coagulation time under temperature and hematocrit variation[35, 36].

Red blood cell aggregation is also a sensitive inflammation marker[37]. RBCA determination from erythrocyte sedimentation rate(ESR) is extensively used, but it proves unspecific unless corrected for hematocrit(Ht)[38]. Moreover, whole blood viscosity measurement at low shear rate is also sensitive to RBCA but is cumbersome to apply. Electrical impedance of blood (sensitive to spatial RBC distribution) was used to determine RBCA in low shear conditions[39]. Shape and rigidity effects under different flow conditions were analyzed by studies combining both hemorheology (controlled shear flows) and electrical impedance assays[40].

Whereas physiological properties of human erythrocytes in inflammation is an intensively studied topic, as recently highlighted[41], the mechanism behind erythrocyte involvement in acute inflammation is not fully understood. As indicated in a couple of very comprehensive reviews[41,42], sepsis induces profound changes in microcirculation with loss of capillary density, as well as by alterations in blood rheology resulting from decreased RBC and WBC deformability, RBC aggregation and coagulation disturbances. Moreover, sepsis relates to morphology changes, e.g. septic RBCs were found to be more spherical[43] as opposed to the characteristic biconcave disc shape, as

shown in ***Fig. 2*** (second row).

SHELF LIFE OF BLOOD

Assessment of donor blood freshness (storage alterations) is another highly important biomedical issue that is currently supported by electrical impedance assays[44,45]. Monitoring the electrical impedance of blood during several weeks of storage at 4 ℃[46] revealed a decrease of both plasma resistance and the capacitance of cell membranes, while the resistance of the RBC interior fluid did not change significantly. These alterations are consistent with RBC lesions during storage and indicate that electrical impedance measurements are useful for monitoring RBC ageing and assessing the quality of stored RBCs. The membrane capacitance is a convenient parameter, allowing a quantitative measure of membrane state that can be otherwise studied with more complicated methods, such as electron microscopy and chemical analyses. Biomedical applications are supported by the differences in membrane capacitances of tumor tissues and of haemolyzed blood, which might be useful for evaluating the biocompatibility of blood related biomaterials[47]. Moreover, the shape of human red blood cells deteriorate progressively during hypothermic storage, with echinocytosis being the most prevalent pathway of this morphological lesion[48]. The change in shape of RBCs from normal discocytes progressively through various stages of echinocytosis to spherocytes produced a substantial decline in the ability of these cells to perfuse an artificial microvascular network[49]. Echinocytosis induced by hypothermic storage could therefore be responsible for a similarly substantial impairment of deformability previously observed for stored RBCs[48]. Shape was proven to be essentially related to the decrease in the velocity difference between the cell and imposed flow, thus providing higher flow efficiency for RBCs. Higher membrane rigidity leads to a dramatic change in the slipper morphology, thus offering a potential diagnostic tool for cell pathologies[50].

HINTS ON BLOOD CELL RELATED DISEASES-RBC INFECTIONS (E.G. MALARIA), CHRONIC FATIGUE SYNDROME, AND INHERITED BLOOD CELL DISORDER (ANEMIA)

The electrical properties of biological cells reveal their healthy/pathological states. Electric impedance microflow cytometry (EIMC) can be used to characterize disease states of RBC. Such a platform comprises a microfluidic device for a label-free and non-invasive cell-counting assay through electric impedance sensing[51]. Invasion by *Plasmodium falciparum* induces physical and biochemical changes on host RBCs throughout a 48 h multi-stage life cycle within the RBC[52]. As such, it also induces progressive changes in the electrical properties of host cells. It was demonstrated that the EIMC system in combination with data analysis allows differentiation of *P. falciparum* infected RBCs from uninfected ones, as well as among different *P. falciparum* intra-erythrocytic asexual stages including the ring stage[52].

Recently, a novel electrical impedance test for the diagnosis of a challenging disease i.e. myalgic encephalomyelitis/chronic fatigue syndrome based on disease related blood cell modification was proposed[53].

Sickle cell disease (SCD) is a common inherited blood cell disorder that affects hemoglobin. The disease state of a sickle red blood cell is closely related to the intracellular hemoglobin composition and concentration. A mutation in the β-globin gene changes the hydrophilic glutamic acid to a strongly hydrophobic valine amino acid, resulting in abnormal hemoglobin S(HbS)[54]. At low oxygen tension, HbS polymerizes and forms rigid fibers, giving rise to rigid RBCs with distorted cell membranes, known as cell sickling. These rigid sickled cells adversely affect blood circulation and oxygen transport efficiency, and have been associated with anemia severity and vaso-occlusive manifestations in various sickling syndromes[54]. The standard diagnosis of SCD is based on hemoglobin analysis, which typically requires a hemolysate using a hemoglobin analyzer. An electrically based microflow cytometry method, with oxygen control for the detection of sickle cells was recently proposed; its results indicate that electrical impedance signals are able to differentiate sickle cells from normal cells[54, 55].

BLOOD CELL ANALYSIS FOR MONITORING DISEASE PROGRESSION

$CD4^+$ T-lymphocyte count is a widely used method for monitoring acquired immunodeficiency syndrome (AIDS) progression, staging, and response to drug therapy in human immunodeficiency virus(HIV) infected individuals in resource poor settings. According to WHO guidelines, a $CD4^+$ T-lymphocyte count of fewer than 200 cells/μL in whole blood establishes the diagnosis of AIDS. Single cell microfluidic impedance cytometry has been used to identify cells at high speed, on the basis of their dielectric properties, however it cannot be used to identify subpopulations of cells (there is no electrical analogue to a fluorescent label). For changing the electrical properties of a

target subset of cells, small antibody conjugated beads are mixed with cells to bind to the target population. This method was used to discriminate and quantitate antigenically defined $CD4^+$ T-lymphocyte subpopulation in human whole blood by an electrical impedance assay[56].

Recent advances in the mechanisms of platelet activation and potential applications of platelet activation biomarkers to diagnose and predict disease states were recently reviewed and discussed[57, 58]. Accordingly, several markers of platelet activation have been identified to correlate with the presence of inflammation and atherosclerosis. Since these markers have relatively short detectability in circulating blood, platelet–monocyte aggregates have recently emerged as markers for platelet activation[59].

CONCLUDING REMARKS

The electrical impedance (or related dielectric/permittivity) spectra of blood can provide relevant biomedical information. When using simple models (e.g. equivalent circuit, as in ***Fig. 1***) one can derive plasma resistance as well as membrane capacitance that directly correlate with the erythrocyte sedimentation rate and can be also used as valuable quantitative tool to assess the quality of stored blood. More intricate (e.g. microscopic) approaches can reveal RBC shape as well as quantitate RBC aggregation (both rouleaux and clot formation) with high biomedical relevance.

Electrical impedance assays actually measure several RBC parameters, i.e. concentration, size, electrical properties to assess cell aging, in particular Rp (plasma resistance) and Cm (membrane capacitance), as represented in ***Fig. 1***. As with aggregation and shape (when jointly used with a method able to control cell orientation versus the direction of the electric field), other parameters with biomedical relevance (e.g. deformability, membrane rigidity) require combined approaches with complementary methods involving (micro) fluidics including rheology assays, as highlighted within the cited *Biomicrofluidics and Hemorheology reports*.

Having in view the rather wide medical applicability of EIS data highlighted in the previous section, either per se or in combination with (micro) fluidics in lab-on-a-chip devices, EIS is expected to further evolve to become an ubiquitous effective, non-invasive tool to support rapid diagnosis in point of care units.

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