LABORATORY TESTING OF HUMAN BLOOD SAMPLES USING MICROFLUIDIC ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY

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Purpose

Electrochemical impedance spectroscopy has great potential for laboratory blood tests [1]. This study aims to develop and verify a microfluidic sensor for determining the hematological parameters of blood based on its dielectric properties.

Introduction

- Impedance measured in a microfluidic channel within the frequency range of 40 Hz to 110 MHz
- **Two measurement configurations (Fig. 1):**
  - Using electrodes 1 and 2 for measurements perpendicular to blood flow
  - Using electrodes 2 and 3 for measurements parallel to blood flow
- **Challenges in analysis:**
  - Preferred orientation of erythrocytes in the microchannel
  - Shape changes of erythrocytes under different osmotic conditions
  - Hemoglobin hydration
- **Theoretical approach:**
  - Based on effective medium theory to account for these factors and accurately measure blood hematocrit, erythrocyte size, dielectric properties of blood components, and hemoglobin hydration

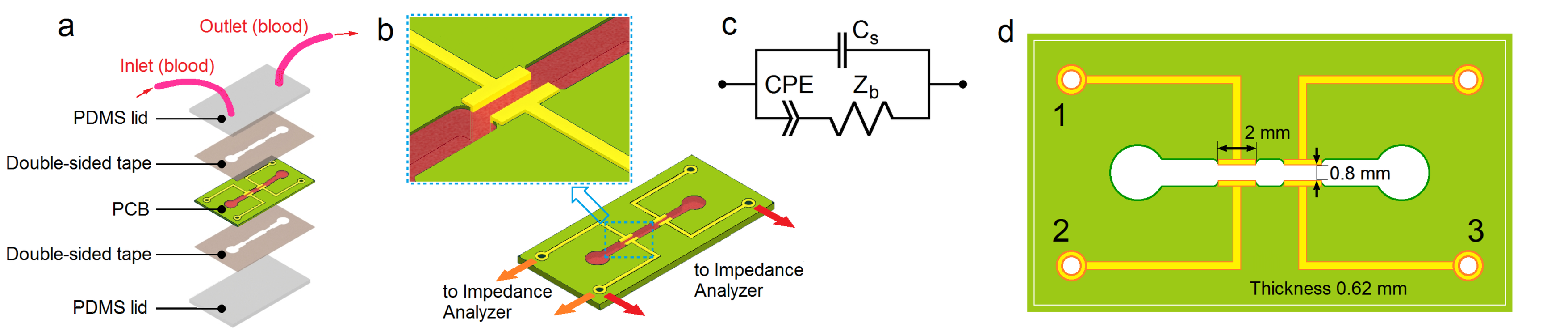

Fig. 1. Schematic of the measuring device. (a) Microchannel assembly. (b) Electrode placement for measuring impedance, showing two pairs of electrodes on opposite walls of the microfluidic channel. (c) Equivalent circuit for impedance measurement in a two-electrode configuration. (d) Detailed layout of the electrode positions in the microchannel.

Materials & Methods

Erythrocyte Alignment and Dielectric Anisotropy in Flowing Blood

- Impedance measured in flowing blood to prevent aggregation and sedimentation
- High volume fractions and shear rates align erythrocytes with the flow direction, causing dielectric anisotropy(Fig.2)
- Anisotropy modeled using the preferred orientation factor (POF)
- Experimental results:
  - 66% of erythrocytes show random distribution
  - 34% are aligned along the blood flow
- No significant changes in POF observed with blood flow rates between 2 and 20 mL/h

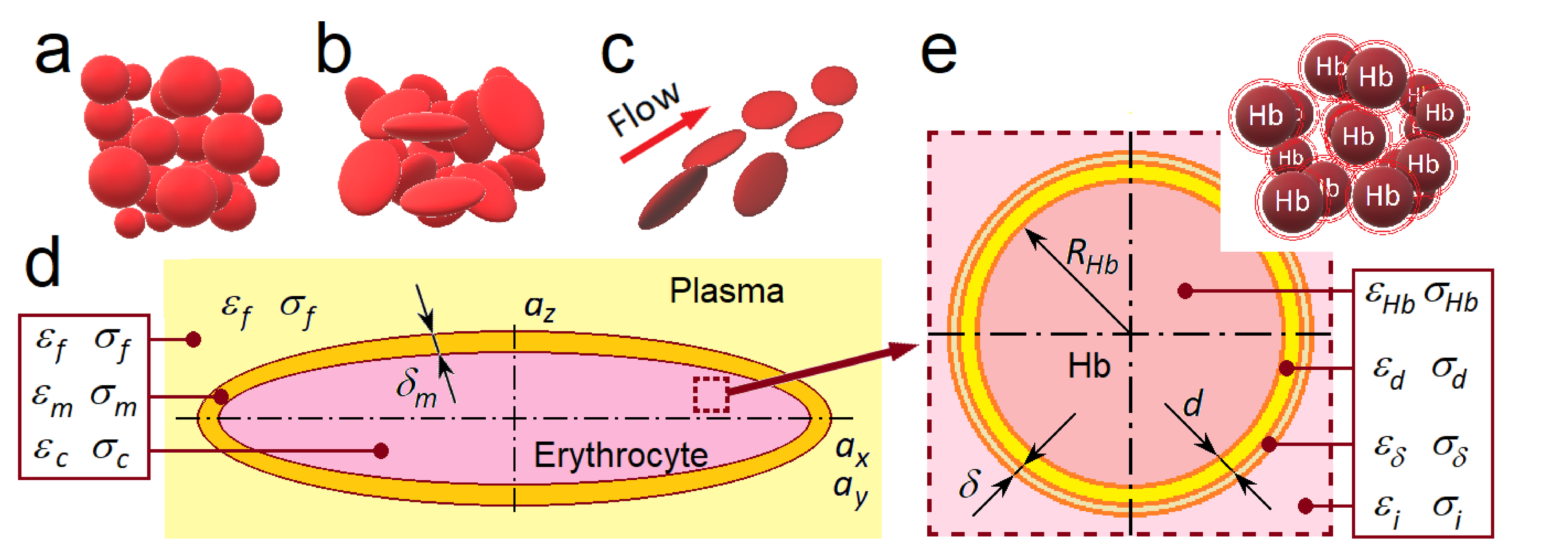

Fig. 2. Models of erythrocyte suspension: (a) random distribution of spherical particles, (b) random distribution of spheroidal particles, (c) uniform orientation in the flow direction, (d) oblate spheroidal model, and (e) hemoglobin core with a double hydration shell (inset: random distribution of hemoglobin molecules in the intracellular fluid).

Erythrocyte Shape and Dielectric Properties Under Osmolality Conditions

- Erythrocytes change shape and dielectric properties under different osmolality conditions (Fig. 3)
- Phosphate buffer saline (PBS) used for isotonic solution (erythrocytes retain biconcave shape)
- Adding salt to PBS induces hypertonic state, causing erythrocyte shrinkage
- Diluting PBS with water induces hypotonic state, causing erythrocyte swelling

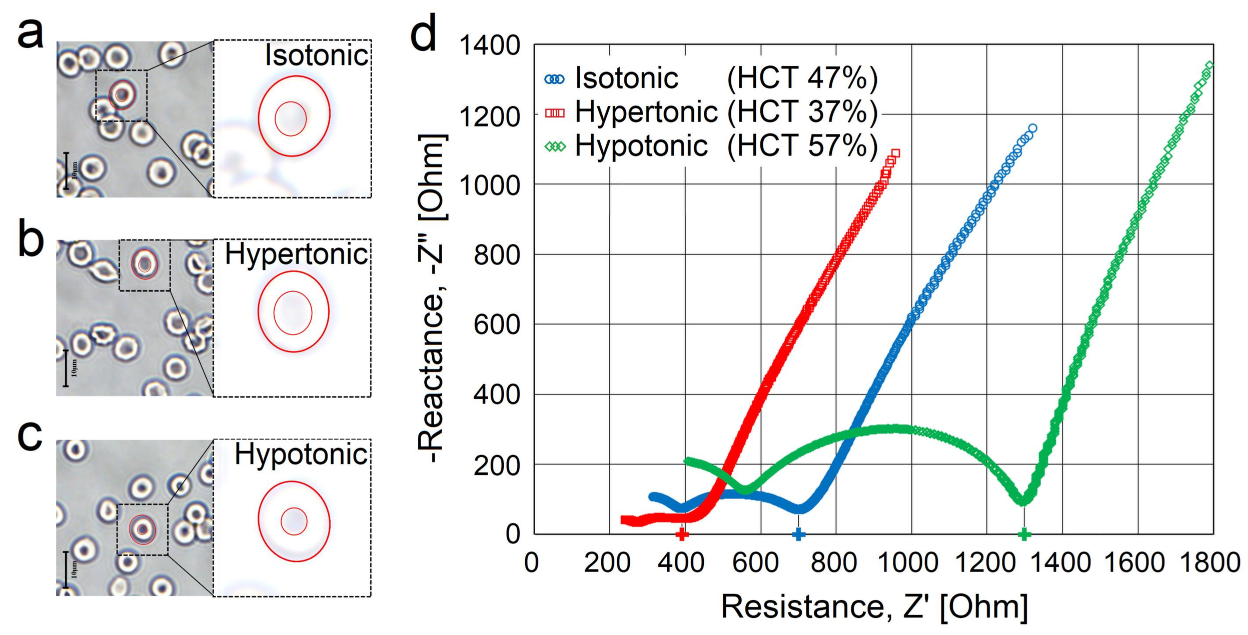

Fig. 3. Blood sample preparation (a). Micrographs of erythrocytes in (b) isotonic (HCT=47%), (c) hypertonic (HCT=37%), and (d) hypotonic (HCT=57%) solutions, and (e) Nyquist plots of changes in blood impedance under different osmolality conditions.

Imaging and Impedance Analysis

- Microscope and CCD camera used to capture images of erythrocytes in various tonicities
- Impedance spectra analysis reveals changes in hematocrit, dielectric properties, and erythrocyte shape

Sensor Sensitivity and Erythrocyte Modeling

- Developed sensor shows high sensitivity to changes in tonicity and erythrocyte shape
- Erythrocyte cytoplasm modeled as a colloidal suspension of hemoglobin molecules with a double hydration shell, improving accuracy of analysis

Comparison of Developed Sensor and Conventional Methods

- Blood samples tested using both conventional laboratory methods and the sensor
- Sample #4 in device EIS-2 used as a representative in Figure 4 to illustrate experimental data and numerical approximation
- Erythrocyte count, hemoglobin level, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) calculated from sensor signals.(Fig. 5)
- Strong agreement with routine laboratory tests (errors less than 5.6%)

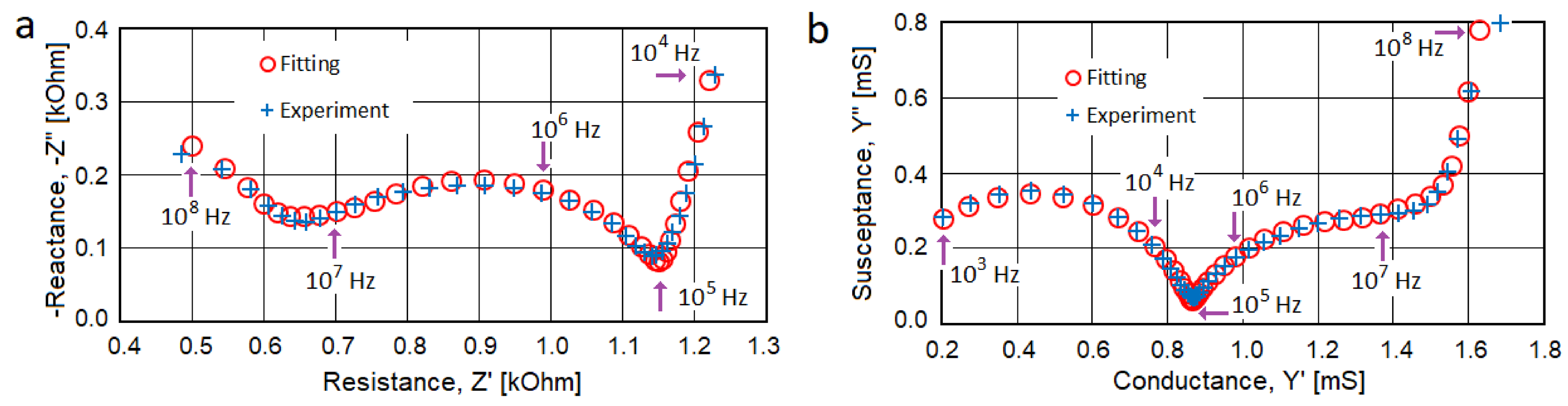

Fig. 4. Impedance (a) and admittance (b) plots for sample #4 in device EIS-2.

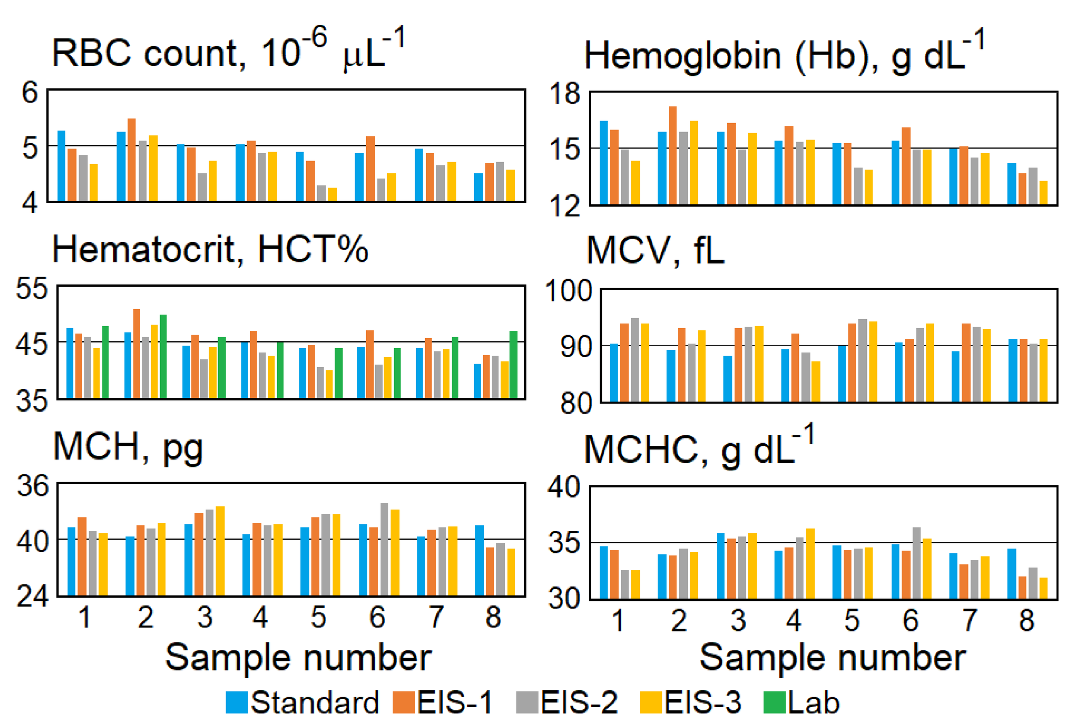

Fig. 5. The RBC count, Hemoglobin level, Hematocrit, MCV, MCH, and MCHC were measured by the standard method and by analyzing impedance spectra using the EIS1, EIS-2, and EIS-3 micro devices. Additionally, HCT was also measured using a micro-hematocrit centrifuge in the lab.

Conclusion

These results provide a new method for laboratory blood testing and could become a valuable tool for point-of-care diagnostics.

Reference

1.A. Zhbanov, S. Yang. *Anal. Methods* 9, 3302 (Jun. 2017)
2.A. Zhbanov et al. *Electrchim Acta* 438 (Jan. 2023)

Acknowledgement

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