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Electrical Impedance of Human Blood with and without Anticoagulants in the β-dispersion Region

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Abstract— **Impedance spectroscopy of blood with and without anticoagulants (Acid Citrate Dextrose (ACD), Ethylene Diamine Tetra-acetic Acid (EDTA) and Lithium Heparin (LH)), taken from healthy donors, ages 24-33, are performed in the frequency range 100 kHz-1MHz, using the two electrode impedance measurement method. High frequency data are used in fitting the Cole plot, assuming electrode polarization effects are negligible. It is verified that this is acceptable since the characteristic frequency of the blood is around 1MHz. Cole parameters are used to evaluate the effects of anticoagulants on blood impedance. Interior resistance of red blood cells is not influenced by addition of anticoagulants, whereas plasma resistance, characteristic frequency and depression angle changed. ACD decreases plasma resistance and alpha value of blood, but increases its characteristic frequency. LH significantly increases plasma resistance, but its effect in the characteristic frequency is not clear. No significant effects of EDTA on the electrical properties of blood are detected.**

I. Introduction

Electrical impedance spectroscopy is a widely used method to model living tissue characteristics by investigating their electrical properties [1]. Results of a recent study indicated that complex electrical impedance measurements might provide a useful method for investigating storage lesions and for tracking the quality of red blood cells under storage by monitoring Cole parameters only [2].

Electrical properties of tissues have been studied since 1800s and there are several reviews about the methodology [3-5]. The tissue is modeled as in Fig. 1, a capacitive cell membrane with conductive extracellular and intracellular fluids.

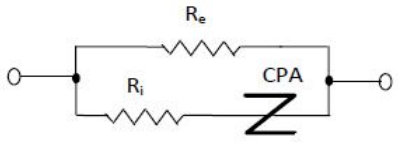

Figure 1. Electrical model of tissues.

As in many biological tissues, plot of the real versus the imaginary components of the measured impedance (Z = R + jX) of blood results in a semicircle whose center is below real axis. The depressed semicircular locus is called Cole plot and modeled by the following Cole equation [6]:

$$Z^* = R_\infty + \frac{R_0 - R_\infty}{1 + \left(j\omega\tau\right)^{1-\alpha}} \quad (1)$$

The intersections of the Cole plot with the real axis provide the dc resistance value R_0 and the infinite frequency resistance, R_∞. R_0 is equal to plasma resistance (R_e) and R_∞ is given by the parallel combination of R_e and the red blood cell interior resistance (R_i). Capacitive effects of cell membranes are usually expressed in constant phase angle impedance Z_{CPA} defined by:

$$Z_{CPA} = K\left(j\omega\right)^{\alpha-1} \quad (2)$$

where K is a constant, $j=\sqrt{-1}$, $0 \le \alpha \le 1$, indicating degree of depression of the center of the circle from the real axis, and τ ($1/2\pi f_c$) is the relaxation time constant. The frequency at which the reactive component of the measured sample impedance reaches the maximum value is called the characteristic frequency (f_c). Frequency range from 1 kHz up to several MHz is called β-dispersion region. Both extracellular and intracellular components of tissue contribute to the measured impedance in this frequency range [7, 8].

The goal of this study is to determine the effects of anticoagulants on the electrical parameters of human blood. Cole parameters, namely the resistances R_∞ and R_0, characteristic frequency f_c, and α are used to model electrical characteristics of blood in the frequency range called β-dispersion region.

II. Methods

A. Blood Samples and Blood Collection Tubes

Blood samples are obtained from 10 healthy human donors (8 males, 2 females) between ages 24-33, over a hematocrit range of 35.9% to 47.4%. Each subject's blood is drawn into 10 ml Becton Dickinson Vacutainer (BD) tube coated with LH (Lithium Heparin), 8.5 ml BD tube containing 1.5 ml ACD (Acid Citrate Dextrose), 10 ml BD tube coated with EDTA (Ethylene Diamine Tetra-acetic Acid), and 10 ml BD CAT (Clot activator) coated tube without any anticoagulant. CAT coated tubes are used to isolate plasma and the separation time is 60 min [9]. Since

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impedance measurement is done immediately following the donation, usually less than 4 min, the separation of plasma in CAT coated tube during the measurement is assumed to be negligible. Blood sample volumes used for impedance analysis are 5 ml.

B. The Measuring Cell

The measuring cell is a cylindrical-like plastic tube of 1.45 cm diameter and 14 ml total volume. The cell constant (probe factor) determined with NaCl solutions of known concentrations is 1.13 cm^{-1} [10].

C. Impedance Measurement

For complex impedance measurements, HP 4284A LCR meter is used with a constant voltage source level at 1 V. Real and imaginary components of the impedance are measured at room temperature, at multiples of 100 kHz, in the frequency range 100 kHz-1MHz. LCR meter offers six-digit resolution on every range and a measurement accuracy of nearly ±2.1 % ohms in R and X values at characteristic frequency which is around 800 kHz.

D. Cole Fitting

Real (R) and imaginary (X) components of the measured impedance is plotted using *circfit* function on *Matlab* [Fig.2]. Cole parameters are then statistically analyzed to detect differences between blood samples. In this study, whole blood samples are considered; therefore the hematocrit range is very narrow: 35.9% to 47.4%.

III. RESULTS

A. Cole Plots

All blood samples, i.e. erythrocyte suspensions of human donors are plotted on Cole circles separately. Impedances from ten frequencies are used to fit Cole plots and the curves have rms error less than 1.05. R_e and R_i are calculated from R_0 and R_∞. f_c and alpha value are calculated with commonly used 'ln (u/v)' method [11]. In Fig. 2, a typical example of Cole plot from Donor 1 is seen.

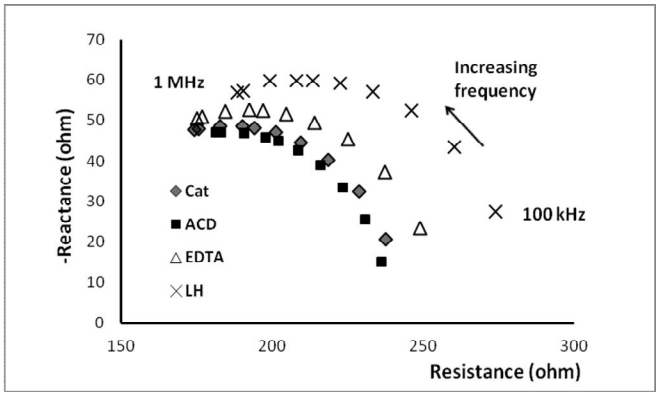

Figure 2. Cole diagrams of Donor # 1. Frequency spectrum is from 100 kHz to 1 MHz, with 100 kHz increments.

B. Cole Parameters

Fig. 3 and Fig. 4 show plasma resistances and characteristic frequencies of blood samples, respectively. Normalization is necessary on resistance to exclude the effect of hematocrit. Since R_e is almost directly and R_i is inversely proportional to hematocrit, normalization is done by calculating $R_{e\,nor} = R_e/Ht$ and $R_{i\,nor} = R_i.Ht$ [12].

		Cat Coated		EDTA		ACD		LH	
	Ht	R_e (ohm)	R_e/Ht	R_e (ohm)	R_e/Ht	R_e (ohm)	R_e/Ht	R_e (ohm)	R_e/Ht
Donor 1	42.9	245.86	5.73	259.95	6.06	240.78	5.61	286.69	6.68
Donor 2	47.4	239.00	5.04	250.14	5.28	234.89	4.96	274.29	5.79
Donor 3	42.9	230.26	5.37	215.08	5.01	202.21	4.71	237.60	5.54
Donor 4	41.4	229.83	5.55	229.60	5.55	215.60	5.21	245.36	5.93
Donor 5	44.9	262.69	5.85	247.22	5.51	230.29	5.13	266.09	5.93
Donor 6	35.9	180.84	5.04	191.14	5.32	177.97	4.96	201.38	5.61
Donor 7	41.5	241.34	5.82	236.45	5.70	211.57	5.10	259.01	6.24
Donor 8	39.6	215.63	5.45	214.24	5.41	194.63	4.91	228.01	5.76
Donor 9	39.1	235.54	6.02	218.62	5.59	214.39	5.48	250.49	6.41
Donor 10	39.9	238.10	5.97	226.78	5.68	215.27	5.40	235.30	5.90
	avg	231.91	5.58	228.92	5.51	213.76	5.15	248.42	5.98
	std	21.64	0.36	20.41	0.28	18.98	0.28	24.70	0.36

Confindence Interval for Re

Cat Coated		EDTA		ACD		LH	
216.43	247.38	214.32	243.52	200.19	227.34	230.75	266.09

Figure 3. Plasma resistances of blood samples from 10 donors.

	Cat Coated	EDTA	ACD	LH
Ht	f_c (kHz)	f_c (kHz)	f_c (kHz)	f_c (kHz)
Donor 1	42.9	718.24	652.32	871.84	624.22
Donor 2	47.4	824.22	740.22	1029.1	750.32
Donor 3	42.9	896.61	836.05	1136.7	813.69
Donor 4	41.4	784.06	775.02	937.51	736.27
Donor 5	44.9	763.79	756.03	955.24	705.25
Donor 6	35.9	1181.2	1028.9	1097.3	975.09
Donor 7	41.5	822.61	827.82	1118	765.95
Donor 8	39.6	790.12	778.87	1022.3	770.47
Donor 9	39.1	677.54	835.36	939.67	671.71
Donor 10	39.9	855.66	772.5	1042.4	767.09
avg		831.41	800.31	1015.01	758.01
std		138.37311	97.10319	87.522309	93.80095

Confindence Interval for fc

Cat Coated		EDTA		ACD		LH	
732.43	930.38	730.85	869.77	952.40	1077.61	690.91	825.10

Figure 4. Characteristic frequencies of blood samples from 10 donors.

IV. STATISTICAL RESULTS AND DISCUSSION

Friedman test is performed on R_e, R_i, f_c and alpha to determine differences in impedance of blood including different anticoagulants. Although normalization is necessary on R_e (R_e /h) and R_i (R_i.h), it did not changed statistical results. Student-Newman-Keuls (SNK) test is performed for pair-wise comparisons (Table 1).

ACD and LH significantly influence plasma resistance but no significant effect of EDTA on extracellular resistance is detected. On the other hand, f_c changes when any of three anticoagulants is added to blood. Red blood cell resistances as indicated in previous studies do not change with addition of anticoagulants [10, 13]. Depression parameter alpha is only different in ACD included blood. Since the amount of ACD in vacuum tube is high (1.5 ml), hematocrit value

significantly reduces in these blood samples. Even in some experiments, not included here, blood samples with low hematocrit value (< 35%) did not show biological tissue characteristics, i.e alpha<0.

TABLE I. COMPARISON BETWEEN ANY TWO OF THE TUBES. NS:NO SIGNIFICANCE, **: P<0.01. *: P<0.05.

	ACD	EDTA	LH	ACD	EDTA	LH
	Re/h			*Ri*h*		
CAT	**	NS	**	NS	NS	NS
ACD		**	**		NS	NS
EDTA			**			NS
	fc			α (alpha)		
CAT	**	*	**	*	NS	NS
ACD		**	**		*	*
EDTA			**			NS

Confidence intervals are also determined for normalized plasma resistance, characteristic frequency and alpha values (with $p<0.05$) to compare blood samples in CAT coated tube without anticoagulant and samples including anticoagulants. It is seen that, ACD decreases plasma resistance between 0.13-0.74 ohms, whereas LH increases it between 0.05-0.73 ohms. The upper values are larger than the standard deviations of normalized plasma resistances changing between 0.28 and 0.36 ohms. ACD increases f_c between 74.8 kHz and 292.4 kHz, with an upper limit higher than standard deviations of f_c. In case of EDTA and LH, although SNK test indicates changes in f_c and alpha ($p<0.05$), however, confidence interval analysis rejects any difference between CAT, EDTA and CAT LH. ACD decreases alpha value between 0.003 and 0.006, again the upper limit is higher than the standard deviations of alpha.

Impedance spectroscopy method is used to characterize the electrical properties of blood with and without anticoagulants. Anticoagulant effects the impedance characteristics of blood cannot be ignored when studying blood impedance.

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