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DIELECTRODYNAMICS OF ERYTHROCYTES OF NORMAL AND DISEASED HUMAN BLOOD

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Abstract: The paper reports the data on percentage variation in dielectrodynamic collection rate (DCR) and threshold voltage (V_{th}) of normal and diseased human erythrocytes. The blood is drawn from healthy persons and from the persons suffering from different diseases. The diseases taken up for the study are Thrombosis, Malaria, Jaundice, Diabetes Mellitus and Cancer. The present study demonstrates that the physiology of erythrocyte membrane is influenced due to disease. The erythrocyte seems to behave as a very sensitive sensor to pick up signals and store them in its membrane due to which it may become more dielectric (in Cancer) or less dielectric (in Malaria, Thrombosis, Jaundice and Diabetes Mellitus) than normal erythrocyte. The dielectrodynamic investigation suggests that the human erythrocyte physiology is perturbed due to disease and these perturbations in cell physiology are mirrored in DCR and threshold voltage spectra. Apart from characterizing the blood cells, the dielectrodynamic investigations could also be extended for monitoring the disease.
Keywords: Dielectrodynamics, NUEF, Dielectrodynamic Collection Rate (DCR), Threshold Voltage (V_{TH}), Human Erythrocytes, Disease.

1. Introduction

Dielectrodynamics or Dielectrophoresis is the translational motion of the matter caused by the polarization effects in a non-uniform electric field (a.c or d.c). Non-uniform electric field can be generated by employing different field geometries.

The first application of non-uniform electric field effects on biological matter or in other words biological dielectrophoresis was described by Pohl and Pylmale [1]. The separation of living cells from dead was made by Pohl and Hawk [2]. Mason and Twonsley [3] made use of DEP technique to separate living cells and organelles of the same species that differ only in diet. Chen and Pohl [4] described a technique known as single cell dielectrophoresis to find excess permittivity of a single cell. Pohl [5] characterised single cell organism and organelles by their characteristic yield spectra. Eisenstadt and Schienberg [6] determined the diffusion coefficient of proteins in solution. Pohl and his collaborators made dielectrophoretic analysis on biological matter at cellular, particulate and molecular levels.

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Gopala Krishna et al [7, 8] reported the dielectrophoretic collection rate (DCR) of yeast cells *saccharomyces cervisiae* considering cylindrical field geometry. The excess permittivity of individual yeast cells was determined by subjecting them to both dynamic and static single cell dielectrophoresis [9].

The behaviour of erythrocytes belonging to animals of different locomotion was studied by Gopala Krishna et al [10], using dielectrophoretic spectroscopy by subjecting them to spherical field geometry.

Ram Mohan [11] carried out studies on comparative dielectrophoretic nature of erythrocytes belonging to human, mammalian and aves. He characterized erythrocytes of different animals having different locomotions, morpho-physiological conditions and metabolic activities.

Dielectrophoretic collection rate (DCR) of human, frog, chicken and pigeon erythrocytes was determined by Gopala Krishna et al [12], using spherical field geometry. The differences in DCR spectra were attributed to the variations in electrical make up of the cell.

Gopala Krishna et al [13] investigated the influence of physical variables such as the frequency, voltage of the applied electric field, suspension conductivity, cell concentration and exposure time of the cell to the non-uniform electric field on DCR spectra of human erythrocytes by employing cylindrical field geometry in the frequency range of 3 kHz - 1.5 MHz. Their results were in the conformity with the theory of dielectrophoresis.

Gopala Krishna et al [14] reported excess dielectric constant for mitotic and non-mitotic *S. cerevisiae* cell in the frequency range of 100 Hz to 20 MHz using single cell levitation technique. Signified differences in characteristic frequencies of DEP spectra were attributed to variations in molecular composition and to the cellular electric fields.

Gopala Krishna et al [15] reported the results of systematic dielectrophoretic study on human erythrocytes of A, B, AB and O blood groups treated with citrate phosphate dextrose-adenine (CPD-Adenine), as an anticoagulant, in the frequency range of 0.1 MHz to 10 MHz using spherical field geometry under a set of experimental conditions. The results demonstrated that the DCR versus frequency is group specific.

During electrofusion, Stenger et al [16] calculated the total pressure acting normal to membranes of closely positioned pronase treated human erythrocytes. The total pressure was modeled as the sum of pressures arising from membrane potential and dipole-dipole attraction opposed by inter-bilayer repulsion.

Huang and Pethig [17] designed special electrodes for negative and positive dielectrophoresis. Gopala Krishna et al [18] established the alteration in electrical double layer structure in yeast cell using dielectrophoresis (cell levitation technique). *S. caerevisiae* at different phases of cell cycle were subjected to dielectrophoresis and variation in excess permittivity at two spot frequencies (10 kHz) in α-region and (1 MHz) in β-region were obtained. They explained the results in terms of electrical double layer structures, biochemical composition and cellular electric fields.

Gopala Krishna et al [19] reported the influence of thrombosis on dielectrophoretic collection of human erythrocytes. The dielectrophoretic collection rate (DCR) of normal and diseased human erythrocytes was studied by varying the frequency of applied field from 1 MHz to 10 MHz, keeping all other parameters constant. Characteristic frequencies were measured from dielectrophoretic spectra.

Dielectrophoretic characterisation of normal and diseased blood was reported by Gopala Krishna et al [20]. The dielectrophoretic behaviour of human red blood cells (HRBC) from patients suffering from diabetes mellitus and jaundice was observed. The dielectrophoretic collection rate (DCR) of HRBC was studied in the β -dispersion region keeping all other parameters constant by applying spherical field geometry. Unique frequency dependent spectra, characteristic of HRBC physiology have been obtained for normal and diseased cells examined under identical conditions.

Gopala Krishna et al [21] studied human red blood cells (HRBC) of normal and leukemic blood by using the principle of dielectrophoresis. The threshold voltage for the dielectrophoretic collection of the normal and diseased HRBC collected from healthy persons and patients suffering from leukemia were reported, in the β- dispersion region under the action of non-uniform electric field set by spherical field geometry. Significant variation was observed in the threshold voltage for collection of normal and cancer samples at the same frequencies under identical conditions of the experimentation.

Jafer Sadiq et al [22-24] developed mathematical models for the calculation of dielectrophoretic collection rate (DCR) and excess permittivity (K_e) of human erythrocytes for the techniques of dielectrophoretic collection rate and single cell dielectrophoresis. They showed the agreement between calculated and experimental values. However, in the past, the DCR and K_e values obtained from Pohl's theory were in agreement with experimental values.

Khan Tabassum Tanweer, et al [25] reported the data on Dielectrophoretic collection rate (DCR) and Threshold Voltage (V_{th}) of erythrocytes of healthy persons and patients suffering

from thrombosis. In this study, erythrocyte suspension of normal and diseased (Thrombosis) blood is subjected to non-uniform electric field (NUEF) produced by pin-pin electrode configuration. The Parameters DCR and V_{th} are measured at constant cell concentration, frequency and applied voltage. The study reveals significant differences in DCR and V_{th} of erythrocytes of thrombosis patients, when compared with that of healthy persons.

In the present investigation, the effect of different diseases such as Thrombosis, Diabetes mellitus, Jaundice, Cancer and Malaria on the physiology of red blood cells by Dielectrodynamic technique has been reported. Through this technique the dynamics of biological cells are studied when they are subjected to non-uniform electric field of different strengths and frequencies. Dielectrodynamics is a very sensitive tool to detect subtle changes in cell physiology in the terms of electrical behavior of cells.

The main aim of the present investigation is to study the electrical properties of human erythrocytes of different physiological conditions- normal and disease, by using the technique of biological Dielectrodynamics and determining DCR and V_{TH} of normal and diseased erythrocytes.

2. Materials and Methods

Fresh samples of normal human blood of different groups and the blood samples from the patients suffering from Thrombosis, Diabetes mellitus, Jaundice, Cancer and Malaria were collected from different hospitals. Ethylenediaminic tetra acetic acid (EDTA) was used as an anticoagulant at the rate of 300µl for 20 ml of blood samples. The Dielectrodynamics studies were carried out within one hour of collection of samples. Red blood cells of normal and diseased blood were isolated from plasma by centrifuging the blood at the rate of 1500 rpm for about 15 minutes. The cells were washed in isotonic glycine-glucose solution (2.1% glycine and 5.5% glucose in the volume ratio of 9:1). The packed cells, when washed, were then mixed with isotonic solution. The concentration of the cells was determined using a red blood cell counting chamber and spectro-colorimeter, with optical density as a guide.

3. Experimental

In the present investigation, erythrocytes were subjected to non-uniform electric field produced by spherical field geometry. A pair of platinum wires of diameter 400 µm was placed 1 mm above the surface of a glass slide in such a way that their axes lie along the same straight line with the grounded tips facing each other and were separated by a distance of 520 µm. the wires were passed through a non-conducting ring of 1 cm internal diameter. When this ring is cemented on a glass slide it forms pin-pin electrode chamber, which can

produce non-uniform electric field, when a. c. voltage is applied between the electrodes. The chamber can hold about 0.3ml of cell suspension.

The electrode chamber was mounted on a conventional microscope stage and observations were made with an eyepiece micrometer marked as100 div/cm, each division corresponds to 10 μm at x10 of the objective. The a. c. signals were drawn from R. F. oscillator. The cell suspension of fixed volume (0.2ml) was dropped into the chamber and the electrical signals were applied between the platinum electrodes for 1 minute for a fixed voltage of 40 V_{rms}. The cells were collected at round tips of the electrodes along the field lines in the form of peal chain due to mutual Dielectrodynamics. The average chain length was measured for 1 minute, which gives yield or Dielectrodynamic collection rate (DCR).

4. Results and Discussion

To investigate the electrical character of the basic unit of life – the cell, a recently developed technique, called Dielectrodynamic technique has been employed. The present investigation is mainly concerned with NUEF and its interaction with human erythrocytes in order to understand how electrical properties of erythrocytes from patients suffering from Thrombosis, Diabetes mellitus, Jaundice; Cancer and Malaria are influenced by the disease. For this purpose, dynamic Dielectrodynamic technique has been adopted. For the first time, the minimum voltage for the collection of erythrocytes at the electrode, called the threshold voltage, has been measured at the frequency of 1 MHz.

The study reveals that the percentage variation in the DCR for Thrombosis, Diabetes mellitus, Jaundice, and Malaria is 69%, 43%, 35% and 51% respectively, less with respect to DCR of the normal at the frequency of 1 MHz, whereas the percentage variation in the case of V_{TH} for Thrombosis, Diabetes mellitus, Jaundice and Malaria is 88%, 50%, 8.6% and 28% respectively, more with respect to the normal at the same frequency (Table 1).

Table 1: Data on percentage variation in dielectrephoretic collection rate (DCR) and threshold voltage (V_{TH}) at 1 MHz for different diseases.

S. No.	Sample	Y_N (µm/min)	V_{TH} (volt)	ΔY%	Δ(V_{TH})%
1	Normal	85.25 ± 5.26	19.33±1.35	-	-
2	Thrombosis	25.80 ± 5.33	36.5±4.9	-69.70	88.80
3	Diabetes Mellitus	48.10 ±13.0	20.17±1.9	-43.50	5.30
4	Jaundice	53.00 ±15.8	21.0±2.6	-35.40	8.60
5	Cancer*	111.90 ± 29.0	10.5±1.09	31.02	-45.60
6	Malaria	24.80 ± 3.61	24.8±3.6	-51.20	28.20

Y: Yeild or dielectrophoretic collection rate (DCR) in µm; N: Normal; D: Disease;
V_{TH}: Threshold voltage in volt; % change in Y, $\Delta Y\% = [(Y_D - Y_N)/Y_N]x100$;
% change in V_{TH} = $\Delta(V_{TH})\% = [\{(V_{TH})_D - (V_{TH})_N\}/ (V_{TH})_N /]x100$

* Types of Cancer – Blood cancer, Breast Cancer, Liver Cancer, Cervix Cancer, Bone Cancer, Throat Cancer, Nose Cancer and Lymphoma with abdominal tumor.

In the case of cancer, the percentage of the DCR for all types is 31% more with respect to DCR of the normal whereas in the case of V_{TH} the percentage Variation is 45% less with respect to normal (Table 1).

The present study demonstrates that the erythrocyte membrane physiology is influenced due to disease. The erythrocyte seems to behave as a very sensitive sensor to pickup signal and store them in its membrane due to which it may become more dielectric (in cancer) or less dielectric (in Thrombosis, Diabetes, Mellitus, Jaundice and Malaria) than normal erythrocytes. So in the language of Dielectrophoresis the disease in sensed at the erythrocyte membrane level and the disease seems to drive the membrane towards the more dielectric and less conductive state or vice-versa.

From the present investigation, the following conclusions are drawn with respect to perturbation in erythrocyte physiology due to various diseases and also with regard to Dielectrophoresis technique.

1. The Dielectrodynamic techniques seems as potential tool to sense subtle changes in electro-physiology of erythrocytes.
2. Dielectrodynamic technique can be used, to some extent in monitoring the treatment of a disease through the Dielectrodynamic behavior of erythrocytes.

3. Any disease that may occur at molecular or cellular or tissue level is bound to perturb the electro-physiology of the erythrocyte membrane.
4. Disease analysis at the cellular level may be possible by cellular Dielectrodynamics.

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