THERAPEUTIC EXPOSURE OF DIABETIC BLOOD USING 532 NM-WAVELENGTH LOW LEVEL LASER THROUGH ELECTRICAL IMPEDANCE SPECTROSCOPY (EIS).

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Abstract

Prior information of blood dielectric properties is important and a valuable tool to examine cell morphological structures, functions and mechanisms of their metabolic systems; however, this is under investigated. The study explores the technique of electrical impedance spectroscopy (EIS) measurements to reveal the proper biostimulation conditions for diabetic blood-low level laser therapy. Dielectric spectroscopy of the diabetic patient's blood was studied using Impedance analyser (Agilent 4294A) within the range of frequency 40 Hz-30 MHz. Blood biostimulations were done using a 532 nm-wavelength solid-state laser (diode-pumped). The impedance characteristics of the irradiated diabetic blood and from proper analysis and observations at 50 mW power output within irradiation duration of 10-15 minutes yield an efficient biostimulation and positively enable rejuvenation and proliferation of the deformed erythrocytes and stimulate healthy blood condition. The irradiated diabetic blood got rejuvenated and had polarization of the electrodes (α -dispersion region) spanned from frequency of 40 Hz until reached 11.7- 11.9 kHz range comparable to the healthy human blood. The stimulation formed a positive result of the accurately biostimulated diabetic blood with significant impedance within the cell structures, cytoplasm and plasma of the blood.

Research Highlights

Electric impedance spectroscopy measurement error increases with dielectric properties variation of the diabetic blood constituents such as; erythrocytes, cell membranes, plasma contents etc. Biostimulation with 532 nm wavelength low level laser, the ionic polarization and dipolar effects were observed within the lower frequency range.

Blood stimulation with 50 mW power output laser within 10-15 minutes, the exposed diabetic blood were noticed to recover positively its morphology structure and physiological conditions therefore became rejuvenated. Higher exposures more than 15 minutes resulted to crenation and formed notch due to excessive heating therefore bio- inhibit and deteriorated.

The low intensity laser stimulation have positive biostimulation effects, increase the ATP synthesis, cell impedance of irradiated diabetic-blood and healthful state as a non-invasive therapy.

Graphical Abstract

Fig. 1: Nyquist plots of a) normal and b) control diabetic blood-impedance with two-electrodes technique with modified Randle's circuit showing various dispersions at different frequency ranges.

Figure 2a) Smeard control non-diabetic blood morphology and **b)** diabetic blood morphology showing bloats RBCs, immature RBCs and some blast cells in blue circle. **Magnifications 40X**

Figure 3a) Smeared diabetic blood irradiated within 10-15 minutes and **b)** smeared diabetic blood irradiated >15 minutes durations showing cell crenation due to excessive radiation, using laser power output of 50 mW. **Magnifications 100X.**

Research Objectives

The work aim is to determine the biostimulation effects of low-level laser irradiation on diabetic blood through dielectric permittivity and its electrical impedance with the help to optimize the laser exposure treatment threshold to avoid bio-inhibition but encourage diabetic blood therapy. The objectives are to use the electrical impedance spectroscopy in studying the dielectric parameters in normal and diabetic blood induced by low level laser. To study the impedance levels of bio-stimulated blood then compare the dielectric response to suggest the appropriate exposure parameters for optimal laser therapy. The significant of the research is to elucidate information that will aid in the future development of non-invasive therapy for the effective treatment of diabetic mellitus (blood) using low level laser.

Methodology

Blood sample collection and physiological examinations.

Total of 84 patients were involved in the study and 8 millilitres each of their blood extacted from Universiti Sains Malaysia (USM), Wellness Centre, Penang, Malaysia under Human Ethics acceptance via JePEM, USM, reference no. USM/JePeM/16060208. The participants included (Group 1 of 42 non-diabetic blood: Males were 27 and 15 Females non-pregnant and Group 1 of 42 diabetic blood: Males were 23 and 19 Females non-pregnant) patients of age range 24-54 years, mean age of 44 old. The glucose level range of the diabetic blood was 7.8-11.3 mmol/L. the collected blood were from patients without serious blood related disease or on medication. A wavelength of 532 nm laser (solid state) with beam spot of 4.0 mm was used for the diabetic blood irradiation on $1.26 \times 10^{-5} \text{ m}^2$ area, intensity of 3968.254 W/m^2 (for 50 mW). The Irradiation was done for durations of 5, 10, 15 and 20 minutes for the irradiated

samples. Designed cuvettes contained the blood samples that measurements were carried out before and after irradiated. The electrodes areas in collected samples was A (0.36 cm^2) each with gap between of d ($= 0.59 \text{ cm}$), volume of blood sample in the conductivity cell was 1 millilitre[2-4].

Spectroscopy Readings and Analysis of Dielectric Parametrs.

An Impedance analyser (Agilent 4294 A) of 40 Hz-30 MHz frequency range was used for the blood impedance measurements [6-7] by connecting the electrodes of the designed cuvettes to it through medium Kelvin clip lead. Records of blood capacitance and loss-energy of the samples were obtained, then further analysis and evaluation for the dielectric properties; dielectric permittivity ϵ' , loss ϵ'' , conductivity and blood impedance.

Results

The tendency of declined impedance with respect to increased in field frequency enables different dispersions behaviors across the erythrocyte membranes and more blood components. The result elucidates the heterogeneous characterization of blood as demonstrated mostly α and β -dispersions at the reduced frequency range as in Figure 1. From Nyquist plots of the normal blood impedance decreased with square root of the frequency within the range of 40 Hz-11.7 kHz, while 40 Hz-17.6 kHz for diabetic blood. This demonstrates effect of the electrode-electrolyte interface called α -dispersion. They exist β -dispersion from the end region of α -dispersion to 10.2 MHz in non-diabetic blood while spanned within 17.6 kHz-11.6 MHz formed pressed-down semi-circle and decreased effective-resistance in diabetic blood. There exists continuous effect of water-electrical charge storage ability in blood sample from the crest of the pressed-down semi-circle to the measured end of the frequency range (30 MHz).

Diabetic blood has high conductivity due to precarious erythrocytes compared to the normal blood. Photo-response of blood components, white blood cells, platelet, blood plasma, and the red blood cells etc, revealed that these erythrocytes are the major photo-receptors of photons on the cellular level[1].

Irradiated diabetic blood within 10 and 15 minutes (Figures 2&3) demonstrated electrode polarizations at 40 Hz-11.7 kHz and 40 Hz-11.9 kHz frequency range respectively. The resulted impedance significantly appreciated with the least observed conductivity responses. There is formidable erythrocyte membrane at ionic polarization and significant impedance mechanisms comparable to the diabetic blood under controlled.

Findings

The blood impedance magnitude decreased with increased frequency range at applied electric field across the erythrocytes membranes and cells cytoplasm.

Diabetic blood stimulated with 50 mW power output for 10-15 minutes were observed with accurate characterization of the impedance level to yield an optimal biostimulation effect. The polarization at the electrodes (α -dispersion region) occurred within 40 Hz to 11.7- 11.9 kHz frequency range equally to that of the normal and healthy blood. The membrane-bound haemoglobin interaction with laser photons rejuvenate and enhanced erythrocytes morphology and buildup cell membrane. This improves blood physiological conditions, appreciable impedance of the cytoplasm and in plasma, overall moderated adequately the glucose concentration therefore prevents the K^+ ion efflux. Exposure beyond acceptable threshold (Figure 3b) initiates mitogenic motivation likewise lessening of lymphocytes consequently obstructs the stimulus mechanisms therefore causes deterioration.

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