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PAPER

PATHOLOGY/BIOLOGY

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Supplementary Pathway for Vitality of Wounds and Wound Age Estimation in Bruises Using the Electric Impedance Spectroscopy Technique

ABSTRACT: Determination of wound vitality and estimation of wound age are central issues in daily forensic practice. The objective of this study was to develop a new and rapid method for determining wound vitality and estimating wound age in bruises using electric impedance spectroscopy. Forty Sprague–Dawley rats (140–170 g) were divided into five groups: group 1 (n = 8): controls, group 2 (n = 8): postmortem bruises, group 3 (n = 8): bruises 1 h before death, group 4 (n = 8): bruises 3 h before death, group 5 (n = 8): bruises 6 h before death. Measurements of the right gluteus maximus muscle were taken at 6, 24, and 48 h after the rats were sacrificed by cervical dislocation. The results from this study indicate that electric impedance spectroscopy is clearly sensitive enough to differentiate between vital and postmortem wound infliction and to determine the survival time after the infliction of an injury.

KEYWORDS: forensic science, electric impedance spectroscopy, bruise, wound age estimation, vitality of wounds, wound

Wound vitality and wound age estimation belong to the classic research field of forensic medicine. The main questions in forensic practice are whether an injury was caused while the individual was alive or during the agonal or postmortem period (wound vitality) and how long the individual survived after infliction of an injury (wound age estimation) (1). Many scientific papers on these two questions have been reported using such techniques as cytochemical analysis of mast cells, time-dependent RNA synthesis, time-dependent expression of ICAM-1, and immunohistochemical detection of chemokines (2–17). However, the majority of scientific studies in this field deal with dermal injuries because of sharp force. Studies on muscle injuries because of blunt force or other types of trauma are almost completely missing. In this study, our purpose is to develop a new method to determine the vitality of wounds and estimate wound age in bruises using an electric impedance spectroscopy technique.

The electrical impedance of tissues at a series of frequencies provides information about the cell population. The impedance spectrum is predominantly influenced by the characteristics and integrity of the population's plasma membranes, cell volumes, and intracellular and extracellular conductivities. Electric impedance is a complex quantity combining resistance as well as reactance, depending on the frequency of the alternating current. Biological tissues have complex electric impedances because they contain components that have both resistive and charge storage properties. Electrical impedance is displayed as real Z′ (resistive component) and Im Z″ (capacitive reactance component). Typical frequency spectra for biological tissues show the real part of the impedance, which is associated with resistive pathways across tissues, and the imaginary part of the tissue, which is associated with capacitive pathways, such as membrane structures. By recording the electric impedance of a tissue over a frequency range (electric impedance spectroscopy), the frequency-dependent electrical and dielectric behavior of the tissue can be determined. Because the electrical properties of biological tissue are related to its physiological and morphological properties, electric impedance spectroscopy is suitable for detection of tissue composition. Electric impedance spectroscopy has been used to diagnose disease, determine the state of organs, measure body water compartments, measure postmortem changes in dielectric properties of haddock muscle, and predict carcass composition (18–31). In the forensic field, Querido (32–36) has used an electric impedance technique to study the postmortem interval. The use of electric impedance spectroscopy characterization is a novel approach to comprehend underlying operative phenomena in a number of material systems, including biological tissues. Many studies so far are preliminary, but they demonstrate that electrical impedance spectroscopy is a promising technical approach. In this paper, we apply for the first time electric impedance spectroscopy to develop a new and rapid technique for the determination of wound vitality and estimation of wound age in bruises.

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Materials and Methods

Electrode Array and Impedance Measurements

A linear array of four electrodes, transmission lines, and terminals were integrated in an insulated polymethyl methacrylate plastic of 2 mm thickness. The electrode was composed of stainless steel syringe needles (18G, 1.2 × 40 mm) clamped in a parallel array approximately 5 mm apart (33). The terminals of the electrodes were connected to a computer-controlled impedance analyzer SI1260 (Solartron, Farnborough, Hampshire, U.K.) in combination with a bioimpedance interface SI1294 (Solartron).

Animal Preparation

Forty Sprague–Dawley rats (140–170 g) were provided by the Animal Center of Xi'an Jiaotong University. The rats were divided into five groups: group 1 ($n = 8$): controls; group 2 ($n = 8$): postmortem bruises; group 3 ($n = 8$): bruises 1 h before death; group 4 ($n = 8$): bruises 3 h before death; group 5 ($n = 8$): bruises 6 h before death. After the rats were anaesthetized with a mixture of isoflurane and oxygen, they were struck vertically on the right buttock from a height of 10 cm using an iron hammer (diameter 3 cm, weight 500 g). The rats were kept in clean cages and allowed food and water and sacrificed by cervical dislocation at 1, 3, or 6 h postinjury. The rats of the postmortem bruise groups (postmortem 0.5 h) were sacrificed by cervical dislocation and were struck vertically on the right buttock from a height of 10 cm using an iron hammer (diameter 3 cm, weight 500 g). The rats of the control group without injury were handled in parallel. The right gluteus maximus muscle of each rat was excised for analysis.

Sample Preparation and Measurements

Each right gluteus maximus muscle was set on a corkboard. The electrodes were inserted vertically into the central region of the right gluteus maximus muscle and fixed by insertion into the corkboard. A SI1260 impedance analyzer (Solartron) was used in this study. The AC output voltage of the system was set at 1.0 V, and the impedance values were measured with a single scan using a frequency range of 100–1000 Hz with 21 frequency points. Electrical impedance was displayed as real Z′ (resistive component) and Im Z″ (capacitive reactance component). Measurements of all the right gluteus maximus muscles were taken at 6, 24, and 48 h after the rats were sacrificed. After each right gluteus maximus muscle was measured, it was placed *in situ* of each rat. All rats were kept at 20°C.

Statistical Analysis

The maximal value of the real part of impedance (Z′) in a single scan using a frequency range of 100–1000 Hz was used for analysis. All data were analyzed by SPSS Software version 11.5 (SPSS Inc., Chicago, IL). Results were presented as the mean ± standard deviation. A p-value of <0.05 was considered significant (30).

Results

The impedance values were measured with a single scan using a frequency range of 100–1000 Hz with 21 frequency points. Electrical impedance was displayed as real Z′ (resistive component) and Im Z″ (capacitive reactance component). Z′ of each sample was analyzed (Figs 1–5). The maximal value of Z′ in electrical impedance was used for analysis. Results are given as the mean ± SD and t-test. The maximal value of Z′ in electrical impedance when measured 6 h after the rats were sacrificed is shown in Table 1. The maximal value of Z′ in bruises inflicted 1, 3, and 6 h before death is significantly lower than in control bruises ($p < 0.01$). The difference between postmortem bruises and controls was not significant. The maximal Z′ value decreases as the time of infliction of the bruise before death increases (Fig. 6). The maximal value of Z′ in electrical impedance measured at 24 h after the rats were sacrificed is shown in Table 2. In all groups where the bruise was inflicted before death (1, 3, and 6 h), the maximal Z′ value was significantly lower than in the control group ($p < 0.05$). The difference between postmortem bruises and controls was not significant. The maximal Z′ decreases as the time between injury and death increases (Fig. 7). The maximal value of Z′ in electrical impedance measured at 48 h after the rats were sacrificed is shown in Table 3. The maximal value of Z′ in bruises inflicted 1, 3, and 6 h before death is significantly lower than in control bruises ($p < 0.01$). The difference between postmortem bruises and controls was not significant. The maximal Z′ value decreases as the time of infliction of the bruise before death increases (Fig. 8).

Discussion

Most studies on the vitality of wounds and wound age estimation have concentrated on dermal injuries because of sharp force (14). Many papers have been reported using techniques such as cytochemical analysis of mast cells, time-dependent RNA synthesis, time-dependent expression of ICAM-1, and immunohistochemical detection of chemokines. Studies on muscle injuries because of blunt force or other types of trauma, however, are almost completely missing (17). In this study, we used electric impedance spectroscopy to develop a new, rapid technique for the determination of wound vitality and the estimation of wound age in bruises.

In the electrochemistry domain, electric impedance spectroscopy is a well-established method for characterizing the electrical properties of materials and their interfaces exposed to electronically

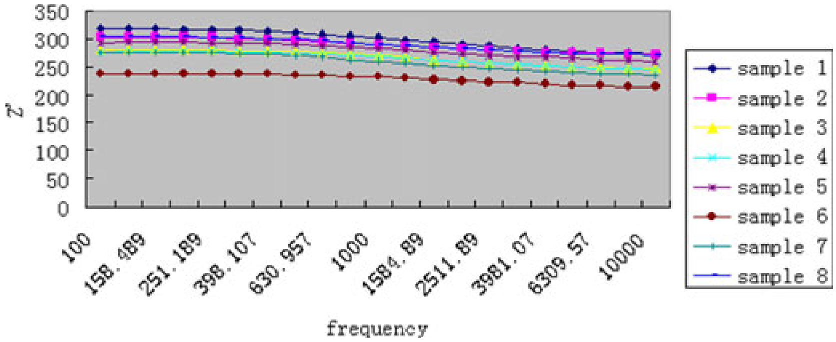

FIG. 1—*Group 1 (*n *= 8): controls (measurement at 24 h after the rats were sacrificed).*

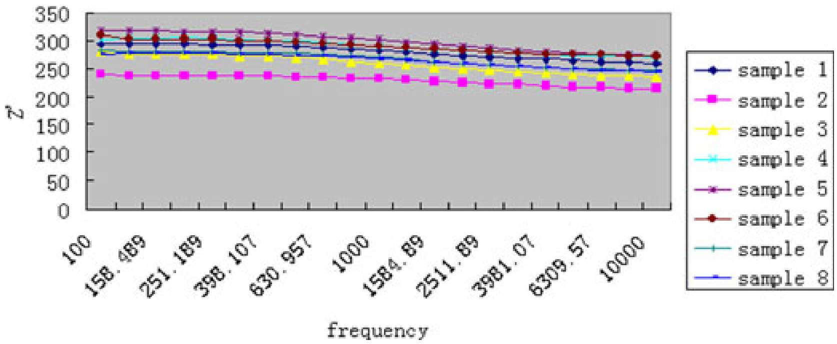

FIG. 2—*Group 2 (*n *= 8): postmortem bruises (measurement at 24 h after the rats were sacrificed).*

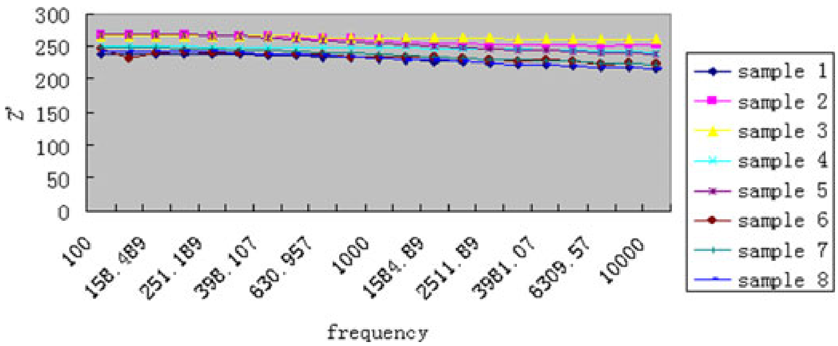

FIG. 3—*Group 3 (*n *= 8): bruises 1 h before death (measurement at 24 h after the rats were sacrificed).*

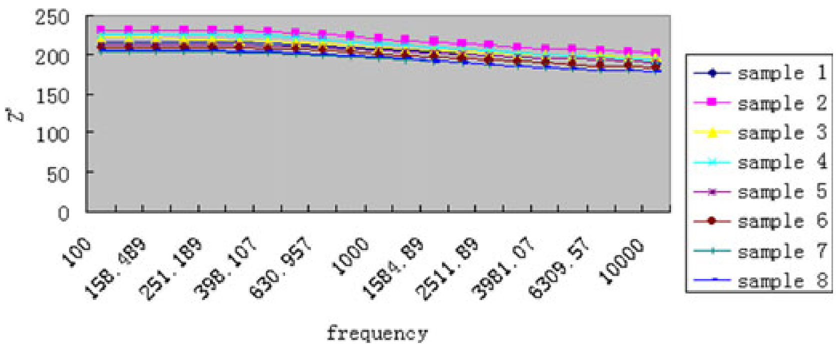

FIG. 4—*Group 4 (*n *= 8): bruises 3 h before death (measurement at 24 h after the rats were sacrificed).*

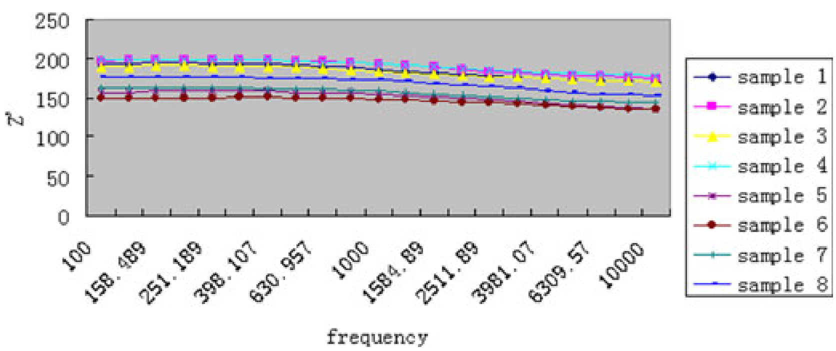

FIG. 5—*Group 5 (*n *= 8): bruises 6 h before death (measurement at 24 h after the rats were sacrificed).*

conducting electrodes. It has been demonstrated that electric impedance spectroscopy can be used as a tool to determine the electrical properties of different heterogeneous systems such as biological membrane/electrolyte systems, including the conductive and capacitive properties of the bipolar membrane junction. Because the electrical properties of biological tissue are related to its physiological

TABLE 1—*Z′ measured 6 h after sacrifice.*

Group	Counts (mean ± SD)	*p* versus Controls
Group 1: controls	375.37 ± 71.79 Ω	
Group 2: postmortem bruises	372.75 ± 67.12 Ω	Not significant
Group 3: bruises 1 h before death	271.12 ± 16.96 Ω	$p < 0.01$
Group 4: bruises 3 h before death	241.62 ± 8.71 Ω	$p < 0.01$
Group 5: bruises 6 h before death	213.75 ± 8.1 Ω	$p < 0.01$

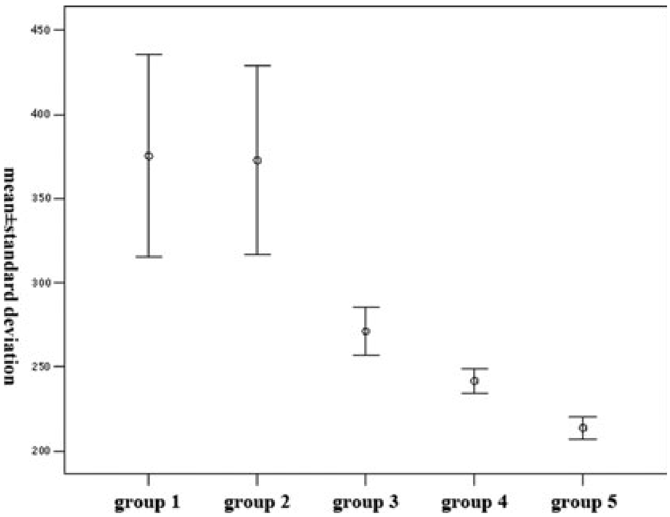

FIG. 6—*Z′ measured 6 h after sacrifice (Group 1: controls. Group 2: postmortem bruises. Group 3: bruises 1 h before death. Group 4: bruises 3 h before death. Group 5: bruises 6 h before death).*

TABLE 2—*Z′ measured 24 h after sacrifice.*

Group	Counts (mean ± SD)	*p* versus Controls
Group 1: controls	285.12 ± 24.54 Ω	
Group 2: postmortem bruises	287.87 ± 25.19 Ω	Not significant
Group 3: bruises 1 h before death	252.87 ± 12.21 Ω	$p < 0.05$
Group 4: bruises 3 h before death	214.75 ± 9.8 Ω	$p < 0.01$
Group 5: bruises 6 h before death	177.75 ± 19.5 Ω	$p < 0.01$

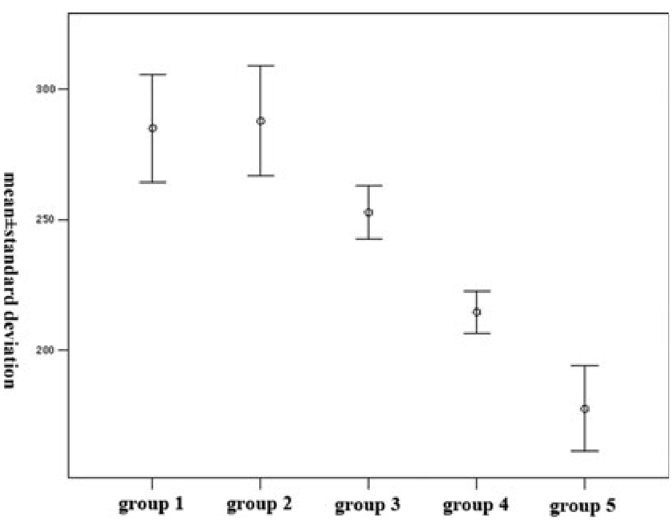

FIG. 7—*Z′ measured 24 h after sacrifice (Group 1: controls. Group 2: postmortem bruises. Group 3: bruises 1 h before death. Group 4: bruises 3 h before death. Group 5: bruises 6 h before death).*

TABLE 3—*Z′ measured 48 h after sacrifice.*

Group	Counts (mean ± SD)	*p* versus Controls
Group 1: controls	178.12 ± 19.34 Ω	
Group 2: postmortem bruises	179.50 ± 19.33 Ω	Not significant
Group 3: bruises 1 h before death	137.25 ± 8.29 Ω	$p < 0.01$
Group 4: bruises 3 h before death	122.25 ± 4.9 Ω	$p < 0.01$
Group 5: bruises 6 h before death	109.00 ± 4.89 Ω	$p < 0.01$

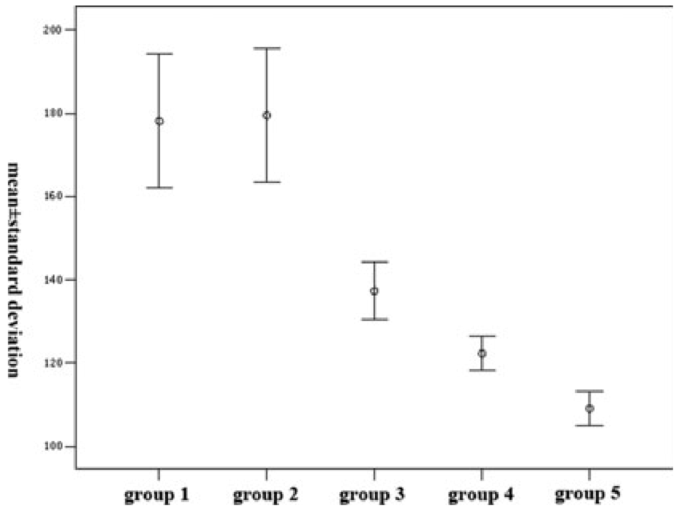

FIG. 8—*Z′ measured 48 h after sacrifice (Group 1: controls. Group 2: postmortem bruises. Group 3: bruises 1 h before death. Group 4: bruises 3 h before death. Group 5: bruises 6 h before death).*

and morphological properties, electric impedance spectroscopy is suitable for detection of tissue composition (30). The use of electric impedance spectroscopy is a novel approach for detection of tissue composition. There are many preliminary studies that show that electrical impedance spectroscopy is a promising technical approach.

In this study, we speculated that the mechanism of electric impedance changes may be related to changes in liquid content in the tissue. When a bruise of the muscles is caused while the individual is alive, the impaired muscles will cause an inflammatory reaction. Therefore, tissue fluid in the impaired muscle tissue would increase. As time progresses, the inflammatory reaction should become more serious, resulting in more tissue fluid in the impaired muscle tissue. Tissue swelling is a cardinal sign of inflammation caused from increased fluid retention that is well measured using impedance spectroscopy. On the basis of the principle of electric impedance, the electric impedance of the impaired muscle tissue would decrease as the liquid content increases. In this study, electric impedance was measured in an animal experiment. Measurements of all right gluteus maximus muscles were taken 6, 24, and 48 h after the rats were sacrificed. All the results displayed that the maximal value of Z′ in bruises inflicted 1, 3, and 6 h before death is significantly lower than in control bruises. The difference between postmortem bruises and controls was not significant. The maximal Z′ value decreases as the time of infliction of the bruise before death increases. The results indicate that this method can be applied in all postmortem stages. The results are consistent with the principle of the electric impedance.

In this process, experimental conditions should be limited to a suitable range: the impedance values were measured with a single scan using a frequency range of 100–1000 Hz with 21 frequency points. The laboratory must be kept at a constant temperature of 20°C because the instrument is sensitive to temperature. The laboratory must also be kept at a certain humidity to prevent the sample from drying. This study is only a preliminary study on animals. Future studies will investigate the correlation between *in vivo* and *in vitro*. We will also conduct a study of body injuries. We expect that our findings can be applied to forensic practice.

Conclusion

Electric impedance measurements were applied in an animal experiment. The results of this study indicate that electric impedance spectroscopy is clearly sensitive enough to differentiate between vital and postmortem wound induction and can determine the survival time after the infliction of an injury. Electric impedance spectroscopy will be a new, rapid, potentially useful tool for determining the vitality of wounds and for estimating wound age in bruises.

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