Conference paper

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In vivo electrical impedance measurement in human skin assessment

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Abstract: Structural and chemical alterations in living tissue are reflected in electrical impedance changes. However, due to the complexity of skin structure, the relation between electrical parameters and physiological/pathological conditions is difficult to establish. The impedance dispersion reflects the clinical status of the examined skin tissue and, therefore, it is frequently used in a non-invasive evaluation of exposing skin to various factors. The method has been used to assess the effect of the fish collagen on the skin of patients suffering from the leg ulcer. Therefore, from a number of different approaches to skin electrical impedance dispersion, the one considered to be safe was selected and applied. This paper presents a short review of different technical approaches to in vivo electrical impedance measurements, as well as an analysis of the results and the effect of fish collagen locally administered on human skin.

Keywords: Chemistry for Beauty and Health 2018; collagen; electrical impedance; leg ulcer; skin assessment.

Introduction

Electrical impedance measurements and impedance analysis are widely employed in biomedical sciences. Electrical impedance is used for the evaluation of the essential parameters of cardiovascular system, such as the cardiac output and systemic vascular resistance, as well as in the case of impedance plethysmography [1]. Analysis of the composition of the human body is becoming more and more popular and is used in clinical practice as well. For example, the bioimpedance analysis of the body composition is safer than the dual energy X-ray absorptiometry (DXA) and demands minimum possible collaboration by the patients. As a result, it is suitable for elderly patients. Bioimpedance analysis (BIA) permits the evaluation of the body components such as

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water, fat tissue and muscle tissue [2]. What is more, the method is used in the nutritional assessment of healthy individuals, as well as in patients suffering from Alzheimer's disease and dementia [3]. BIA is also considered a valuable tool in the evaluation of the hydration status changes in elderly nursing home residents [4]. Additionally, electrical impedance spectroscopy is used in studies on properties of rat lung, other tissues ex vivo [5], as well as the determination of the state of organs [6], whereas electrical impedance is successfully used in the evaluation of skin condition. The skin erythema is a sign of the skin irritation caused by various chemical or physical stimuli and is physiologically derived from vasodilation of local subcutaneous blood vessels where the local excess of water affects electrical impedance of the skin. The clinical qualitative evaluation is based on the visible characteristics and other symptoms. Skin rubor can be identified by means of BIA, since the quantitative method is used in the diagnosis of various skin conditions [7]. Understanding the electrical properties of the skin is also essential in the evaluation of the drugs transport mechanism through the skin, such as iontophoresis.

The skin is the largest organ of the body, and its major functions involve control and protection against the environment. Therefore, its structure and composition are so complex. Its composition is not uniform and varies in the thickness of the stratum corneum, the structure of the dermo-epidermal junction, and the number of glands and hair follicles. Along with differences related to sex, age and race, all these properties can be quantified by means of electrical impedance measurements.

The skin comprises a poorly conducting stratum corneum and the conductive epidermis/dermis, with low and high water content, respectively. Water content level increases from about 20 % of stratum corneum to almost 70 % in the basal cell layer of the epidermis. In subcutaneous fat the water content amounts to approximately 10 % [8].

The stratum corneum (SC) is the primary barrier to chemical penetration, as well as to the passage of electric current through the skin. Due to the complexity typical for biological materials, the relationship between the values of impedance parameters and physiological and pathological tissue conditions is generally difficult to establish through measurements of multi-layer skin structure. What one can conclude here is that generally, as in the case of other tissues, structural and chemical changes in living skin are reflected in electrical impedance changes.

Tissue electrical impedance

Measurements of electrical impedance of human skin, as well as studies of biological systems, are related to direct impedance measurements usually in the areas of frequency and the phase angle. In the impedance measurements, the amplitude of the applied electrical voltage and the resultant current to avoid physiological side effects are important in situ or in vivo [9, 10]. The first published studies on skin impedance were published around 1920. The Bode analysis of skin impedance measurements and phase angle of the skin analysis were applied by Burton et al. [11]. Subsequently, the Cole impedance model was proposed, which was based on replacing the ideal capacitor in the Debye model for a general element called constant phase element (CPE) [12]. The analysis of the Cole impedance model requires three elements: (1) an equivalent circuit; (2) the development of the corresponding equations; and (3) a simulation which gives the complex impedance behavior. Important progress in the analysis of skin bioimpedance was made by Salter [13] and Yamamoto and Yamamoto [14].

Extraction of unknown Cole impedance parameters is still a matter of extensive research; the approach here belongs to either the class of deterministic, or the class of stochastic methods [15]. The analysis of the Cole model for biological systems can be based, on meta-heuristic optimization algorithms for extracting the parameters of the Cole-impedance model [16].

Basic impedance theory

For direct current (DC), this relationship between current I and voltage U is given by the Ohm law:

$$U = RI$$

where R stands for the resistance.

Resistance depends on the geometry of the object, temperature and type of the studied material.

The latter is, characterized by specific resistivity. In AC-circuits, the voltage and current fluctuate harmoniously with a certain frequency. The electrical properties of a sample/object is described by its resistance and capacitance. Therefore, electrical impedance, as the apparent opposition in an electrical current, is considered in terms of two frequency dependent components. This points to the fact that impedance can be represented by frequency dependent magnitude and phase, and for a given frequency it can be represented by the magnitude and phase, two numbers creating a characteristic graph in the complex number space.

What is more, this complex quantity, combining the resistance and capacitance, is dependent on the frequency of the applied AC voltage. Tissue possesses both conductive and charge storage properties. By studying the frequency dependence of electrical impedance of various tissues, its frequency-dependent electric and dielectric behavior can be determined and used for various applications including pathology, prognosis, diagnosis and healing using electrical pulses.

Dispersion of electrical properties

Electrical properties of biological tissues, such as electrical impedance, are dependent on tissue structure and the frequency of the conducted current. Generally, as an extensive thermodynamic parameter, electrical impedance is related to two intensive parameters, namely electrical permittivity and electrical conductivity. Electrical impedance dispersion reveals three major regions of dispersion, as presented by Foster and Schwan [17]. The first one, the α-dispersion region, is present at very low frequencies and is sometimes called "acoustic", due to its frequency range of up to a few kHz; it is associated with tissue interfaces, such as membranes. The second one, found at radiofrequencies (1 kHz up to a few MHz), the β-dispersion region is associated with the polarization of cellular membranes, proteins and other organic macromolecules. This area often contains the more relevant clinical information. The third region, that is the γ-dispersion region, is related to relaxation of water molecule usually spreads into the δ-dispersion (100–500 MHz) and above 1 GHz. Therefore, it is possible to establish that the electrical properties of any biological tissue are dependent on its intrinsic structure. The impedance of the human skin varies in terms of thickness and hydration level, the activity of sweat glands, injuries, patient's age, environmental temperature and humidity.

The human body can be considered a composite heterogeneous volume conductor. It comprises tissues of different electrical properties. Furthermore, electrical conduction within biological tissues depends on ionic concentration, ionic mobility and applied external electric field, which finally produce the ionic current. Bound charges within tissues give rise to complex dielectric properties, and thus displacement currents (a time-varying electric field, contributing to dielectric polarization) which contribute to the time-varying electrical behavior. The above mentioned bound charges include electrical double layers at membrane surfaces and polar molecules, such as proteins. Moreover, conductivity and permittivity vary between biological tissues and depend on frequency. Due to structural complexity, a wide spectrum of relaxation times, as well as a wide spectrum of polarizable entity, each biological tissue exhibits its own specific response to the external AC field.

At low frequencies polarization is maximal, decreasing with the rise in frequency where polarizable entities are unable to react. Thus, dielectric dispersion is associated with biological tissues where the relative permittivity decreases with increasing frequency. Therefore, the cellular components, internal structure and arrangements of the constituent cells, different cellular structures of tissue and geometry of the studied item give rise to characteristic impedance spectra. The knowledge of electrical properties of biological tissues has been crucial for our understanding of their structure and function.

Typical frequency spectra of biological tissues reflect both resistive pathways and capacitive pathways, which are respectively manifested in the real and imaginary part of the impedance.

The real part is large at low frequencies, such as 10 Hz, or lower. Furthermore, the real part of the impedance decreases and the imaginary part becomes more dominant with increasing frequency, and at high frequencies, the imaginary part of the impedance becomes small. Due to the very small time constants at very high frequencies, electrical current does not flow, but ions moves back and forth between membrane surfaces, and hence neither the resistive pathways nor the capacitive pathways of the membranes have time to play a role.

Skin impedance measurement

Electric impedance skin measurements are carried out to test human skin integrity in vitro. In contrast to in vitro testing, in situ or in vivo measurements cannot be expressed in terms of resistivity. The latter is frequently used in the evaluation of permeability coefficient for water, or polar and ionic compounds in skin samples. A correlation between transepidermal water loss (TEWL) and the inverse of impedance (i.e. the admittance) measured at low frequencies was found [18, 19]. Testing skin properties in vitro, listed above, with other frequencies, such as 100 and 1000 Hz was carried out. Electrical impedance has previously been used for the quantification of skin reaction to irritating agents and to assess skin diseases. It is well known that changes in living tissues are reflected in changes of electrical impedance. Nevertheless, due to their complex structure, the relation between measured electrical parameters and the physiological or pathological skin condition is difficult to establish [20].

Electrical impedance spectrometers allow for the measurements of skin properties over a wide range of frequencies, even up to the microwave region, but the most common frequency range covers α- and β-dispersion range of the living tissue resistance, that is the 10-kHz and 10-MHz frequency, respectively. Moreover, the skin also contains dielectric materials, causing the phase shift between applied and resulting signals. Both electrical impedance and phase shift vary with frequency [21].

Measurements of skin impedance were employed in a number of studies on skin reactions. An interesting solution was proposed by Ollmar, who introduced 4 indices characterizing skin impedance at selected frequencies. The idea was to simplify the analysis of skin bioimpedance spectra, as well as to quantify tissue reactions in physiological conditions, as well as in the case of any skin irritation leading to pathological conditions. Moreover, the idea of the coaxial skin probe for the skin bioimpedance measurements was developed and brought into effect. One of the most essential attitudes was the abandonment of the construction of skin electrical equivalent circuit model, and the extraction of skin impedance parameters [22]. According to the author, this technique was at least as sensitive as the measurement of transepidermal water loss and the visual assessment with the naked eye [23]. The skin impedance indices measured at six different anatomical regions (different locations on body surface) had shown significant differences, as well as the day-to-day variations [24, 25]. The method was implemented by other investigators and it was suggested that although the method could discriminate the irritated and non-irritated skin, it does not allow for the discrimination of the irritant substance [26]. The Ollmar approach to skin impedance measurements was rather phenomenological and focused on establishing the standard values of four skin impedance indices and on giving them clinical meaning. In addition, it challenged the necessity to construct an equivalent circuit model. It is comprehensible in the case of the complex structure of skin and, therefore, the complex geometry of the measured in vivo/in situ sample using the open end coaxial probe and four electrodes systems. Another argument in its favour was the limitations of the Cole equations for heterogeneous structures, such as intact skin [22]. In many medical cases, application of the disposable electrodes is mandatory due to patient safety, e.g. Ag/AgCl electrode with adhesive gel in the evaluation of skin rubor caused by the induced vasodilation [7].

Aim: The aim of the paper is skin condition evaluation following the dermal administration of fish skin collagen (silver carp) in leg ulcer by means of electrical impedance measurements.

Materials and methods

The effect of native fish skin collagen administration on the surroundings of the leg ulcer was evaluated by in situ electric impedance measurements. Electric impedance was measured in the frequency range of 500 Hz–2 MHz, following 1 h adaptation at 21 °C, for both the control and the experimental group. The conformation of the collagen molecule used in the experiment was verified by temperature dependence of relative viscosity.

In the study, 59 adults with chronic leg ulcers located on the lower leg area were included in the experiment. Patients were randomized into two groups, the experimental and the control one. The experimental group consisted of 34 patients who had been treated with fish collagen and the standard protocol. Collagen gel contained about 1 % of collagen (dry mass) and was applied into the skin around the ulcer twice a day for 3 months. The condition of the skin was assessed by means of electrical impedance measurements performed at the beginning of the therapy, after 4, 8, 12 weeks of treatment and at 12 weeks after the end of collagen treatment. The control group comprised 25 patients, treated according to the standard protocol, and the skin condition was assessed by this same method, at the beginning of the therapy and after 4, 8, 12 and 24 weeks. Measurements were carried out on both the healthy and ulcerated leg.

Skin electrical impedance, as well as its electrical resistance and capacitance measurements were performed using HIOKI LCR METER IM3536 bridge and Ag/AgCl disposable electrodes. Electrodes were placed on the upper edge of the wound. Electrodes were always placed at this same distance from the ulcer edge in spite of reduced surface of ulcer progressing during the treatment process.

Measurements were carried out at a constant temperature of 21 °C and relative air humidity 40–60 %.

Parameters, such as MIX, RIX and IMIX introduced by Ollmar [22], and defined as ratio of impedances for 20 kHz and 500 kHz, ratio of real part of impedance for 20 kHz and impedance for 500 kHz, and ratio of imagine part of impedance for 20 kHz and impedance for 500 kHz, respectively, were calculated for each measurement.

The Shapiro–Wilk test for normality was performed for all data sets. Wilcoxon and U-Mann–Whitney tests was employed in the case of lack of normal distribution, for dependent and independent variables, respectively, whereas the t-student test was performed for normal distribution. Statistical analysis was performed using STATISTICA 12 by StatSoft.

Results and discussion

The conformation of fish skin collagen was determined by means of the Ubbelohde viscometer, at the temperature range of 20–60 °C. The samples were diluted with distilled water until the concentration of solution was suitable for the capillary viscometer. The sample was heated for 10 min (the incubation time) at each tested temperature range prior to the measurement, or until the flow time by capillary viscometer was stable. The same sample was tested in the whole temperature range. On the basis of the obtained results, the dependence of the relative viscosity on the temperature was established (Fig. 1). The drastic drop in the relative viscosity at the temperature range of 30–40 °C was interpreted as the phase transition related to the thermal denaturation [27] and permitted one to establish the denaturation temperature as (33.4 ± 1.5) °C. A sample heated up to 60 °C (first measurement) was cooled to 20 °C and heated again (second measurement). The lack of the distinct drop in relative viscosity indicated that the total irreversible thermal denaturation of the collagen had occurred during the first heating process.

The indices MIX, RIX and IMIX for each patient were calculated at the beginning of the wound treatment, after 4, 8, 12 and 24 weeks for both the experimental and the control groups. Median values of these parameters are presented on Figs. 2–7, whereas the results of their statistical analysis are collected in Tables 1–3.

Measured values of MIX, RIX and IMIX were also compared to the reference values formulated by Nicander et al. [28]. Because the baseline proposed by the aforementioned authors did not include the results in the lower leg, that is the area where leg ulcers were located and the presented measurements were carried out, the values for the adjacent regions of lower leg, that is the thigh and ankle devised by Nicander et al. were

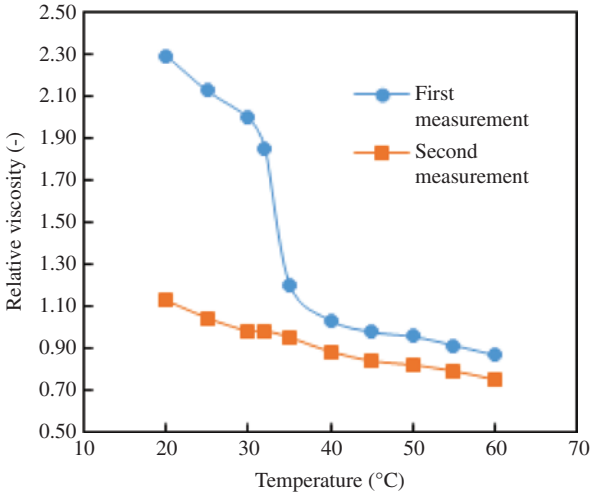

Fig. 1: The relative viscosity of fish skin collagen solution vs. temperature.

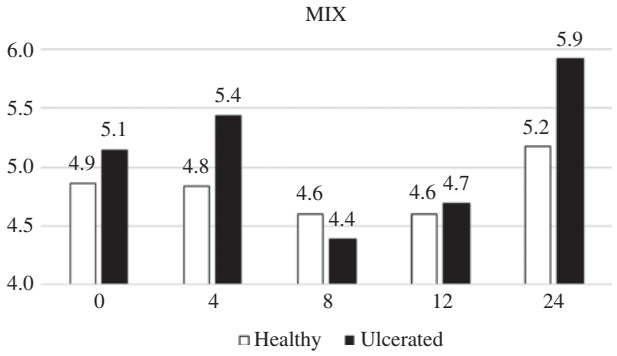

Fig. 2: Median values of MIX parameter for the experimental group measured at the beginning (0), after 4, 8, 12 and 24 weeks for healthy and ulcerated leg.

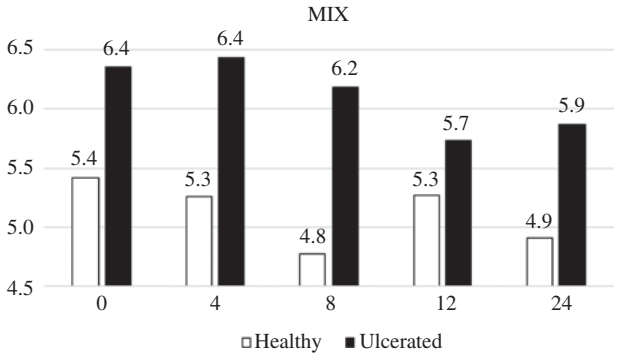

Fig. 3: Median values of MIX parameter for the control group measured at the beginning (0), after 4, 8, 12 and 24 weeks for healthy and ulcerated leg.

taken as reference. Regardless of sex and age, reference ranges were established as follow: 6.79–14.51 for MIX, 2.94–4.17 for RIX and 6.01–14.12 for IMIX [28].

A decrease in MIX with the healing progression was observed for both the experimental and control group. Values came up to a healthy leg results (Figs. 2 and 3). In the final measurement, 12 weeks after the

Table 1: The differences between results of MIX, RIX and IMIX, for healthy and ulcerated leg at the beginning (0), after 4, 8, 12 and 24 weeks.

	0	4	8	12	24
Experimental group					
MIX	0.113781	0.387933	0.469283	0.979541	0.387933
RIX	0.844133	0.168739	0.023805[a]	0.478011	0.725980
IMIX	0.002883[a]	0.003967[a]	0.171661	0.153420	0.011678[a]
Control group					
MIX	0.109386	0.182897	0.191899	0.182897	0.103554
RIX	0.599803	0.840072	0.840072	0.475826	0.475826
IMIX	0.017254[a]	0.047968[a]	0.061481	0.017254[a]	0.008042[a]

[a]Statistically significant difference.

Table 2: The differences between results of MIX, RIX and IMIX for the experimental and control group at the beginning (0), after 4, 8, 12 and 24 weeks.

	0	4	8	12	24
Ulcerated leg					
MIX	0.937771	0.013599[a]	0.000374[a]	0.506939	0.611827
RIX	0.906775	0.211610	0.291896	0.906775	0.667635
IMIX	0.066797	0.016516[a]	0.000125[a]	0.026656[a]	0.969410
Healthy leg					
MIX	0.171858	0.197681	0.093241	0.369278	0.875920
RIX	0.310137	0.274391	0.055779	0.532250	0.937771
IMIX	0.105600	0.112374	0.155933	0.377772	0.575559

[a]Statistically significant difference.

Table 3: Differences between MIX, RIX and IMIX obtained after 4, 8, 12 and 24 weeks of treatment and results before the treatment, in the experimental and the control group, for both ulcerated leg and healthy leg.

	4	8	12	24
Experimental group				
Ulcerated leg				
MIX	0.555305	0.002149[a]	0.139182	0.844133
RIX	0.590205	0.002696[a]	0.342694	0.830776
IMIX	0.479292	0.014687[a]	0.027754[a]	0.628525
Healthy leg				
MIX	0.764796	0.751786	0.993180	0.046400[a]
RIX	0.830776	0.830776	0.979541	0.042772[a]
IMIX	0.844133	0.638245	0.965908	0.068641
Control group				
Ulcerated leg				
MIX	0.798248	0.381860	0.135351	0.367386
RIX	0.339480	0.326050	0.157771	0.396679
IMIX	0.736617	0.562928	0.396679	0.509755
Healthy leg				
MIX	0.657069	0.777543	0.220853	0.509755
RIX	0.544910	0.819095	0.121829	0.411840
IMIX	0.544910	0.676637	0.353259	0.287863

[a]Statistically significant difference.

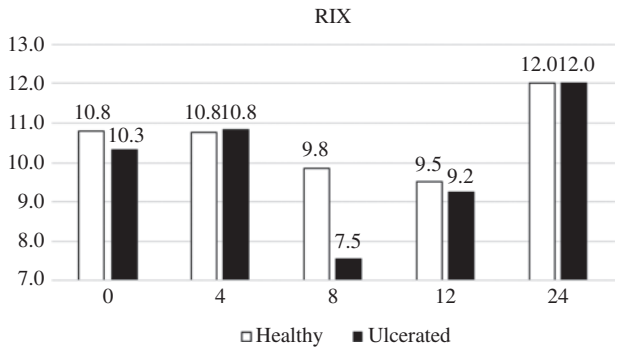

Fig. 4: Median of RIX parameter for the experimental group measured at the beginning (0), after 4, 8, 12 and 24 weeks for healthy and ulcerated leg.

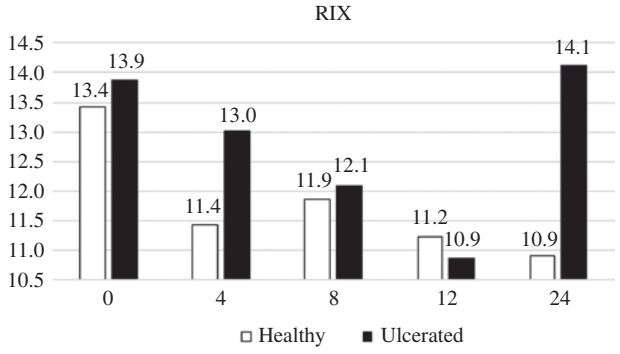

Fig. 5: Median values of RIX parameter for the control group measured at the beginning (0), after 4, 8, 12 and 24 weeks for healthy and ulcerated leg.

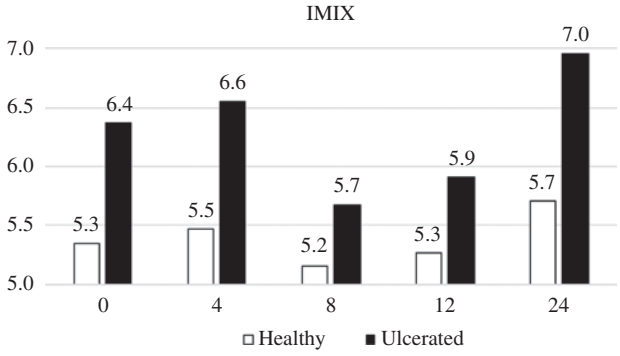

Fig. 6: Median values of IMIX parameter for the experimental group measured at the beginning (0), after 4, 8, 12 and 24 weeks for healthy and ulcerated leg.

completion of collagen application, the increase of MIX parameter was noticed. Changes in MIX values were more distinct in the experimental than in control group. The obtained results in the experimental and control group were lower comparing the reference values range: 6.79–14.51 [28].

The results of RIX for patients with leg ulcers were generally higher than the reference values – 2.94–4.17 [28]. Analysis of RIX values showed rapid decrease of the parameter after 8 weeks of collagen treatment – values are lower than values obtained for healthy skin. Gradual decrease was also observed in control group however changes was slower than in experimental group. Values came up to reference as an effect of collagen administration and healing process. Moreover increased value of RIX can be related to irritant skin reaction

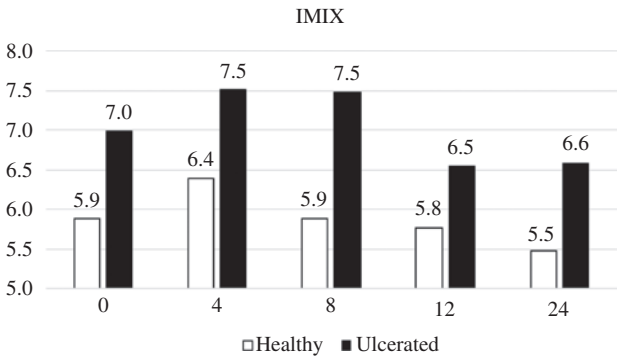

Fig. 7: Median values of IMIX parameter for the control group measured at the beginning (0), after 4, 8, 12 and 24 weeks for healthy and ulcerated leg.

[29], thus it can indicate a positive influence of collagen administration on patients' skin condition. Increase of RIX index 12 weeks after the end of the treatment was noticed in both experimental and control groups.

In the experimental group, IMIX parameter decrease in course of treatment was observed (after 8 week of collagen application), values also came up to healthy leg results. However, in the final measurement IMIX values increased – decline seems to be unsustainable, after the end of collagen application more distinct difference between healthy and ulcerated leg occurred again. An unnoticeable change in IMIX in the control group was found. Moreover our results of IMIX for ulcerated leg are within range of reference formulated by Nicander et al.: 6.01–14.12 [28] whereas, results for healthy leg are generally below aforementioned range of reference.

Values of MIX, RIX and IMIX parameters were compared in the experimental and control group, as well as in both healthy and ulcerated leg (Table 1). In the MIX parameter no statistically significant differences between healthy and ulcerated leg for both experimental and control group were found. In the RIX parameter, for experimental group, the statistically significant difference was found after 8 weeks of treatment. Analysis of IMIX parameter showed significant differences between results for healthy and ulcerated leg for both experimental and control group at the beginning of the therapy (0 week) and 4 week of treatment. A statistically significant difference was also noticed after 24 week, that is at 12 weeks after the end of treatment, also for both experimental and control group. In the control group there is also statistically significant difference after 12 weeks of treatment.

The IMIX parameter seems to be related to the healing process, therefore the improvement of the skin condition can lead to the disappearance in the IMIX differences – skin around ulcer became healthy.

A comparison of the MIX index for the experimental and control groups showed statistically significant difference after four and 8 weeks of collagen application. For a healthy leg there was no statistically significant difference, which is consistent with expectations (Table 2). No statistically significant differences between healthy and ulcerated leg for both experimental and control group, in the RIX index were found.

Significant differences in results of IMIX obtained for experimental and control groups during collagen therapy (4, 8 and 12 weeks) was noticed – that difference can be associated with collagen effect on skin condition. However, in the final measurement no statistically significant difference for IMIX values was noticed – the effect seems to decline after collagen administration has ceased. There were no statistically significant differences noticed for healthy leg, for both experimental and control group, which is consistent with expectations (Table 2).

The results of MIX, RIX and IMIX indices obtained in 4, 8, 12 and 24 weeks of treatment were compared to the results from the first measurement (before treatment) in the experimental and control group, for both ulcerated and healthy leg (Table 3). What is more, a difference between results of MIX obtained at the beginning and after 8 weeks of treatment was noticed. Statistically significant differences occur in the same weeks of treatment (8 and 24 week) for MIX and RIX parameters. For both MIX and RIX index, the statistically significant difference, was noticed after 24 weeks of treatment on healthy leg in the experimental group.

The results (24 week, MIX and RIX) were not consistent with expectations, they will constitute the subject for further analysis.

Moreover, changes in IMIX values after 8 and 12 weeks of collagen application beside the initial results were found. These differences also decreased with the end of the collagen therapy. As expected, in the experimental and control groups a lack of significant changes for healthy leg was also observed.

There were no statistically significant differences in MIX, RIX and IMIX indices noticed in the control group which is consistent with the expectations.

The most visible and statistically significant differences in electrical parameters were noticed after the eighth week of collagen application, which may indicate the strongest influence of collagen treatment on skin condition after 8 weeks. The application of fish collagen precipitates the process of chronic leg ulcers treatment.

Conclusions

The obtained results indicate that measurements of electrical impedance in situ/in vivo can be useful in the assessment of the treatment process for patients with chronic leg ulcers. Determined electrical impedance indices, such as MIX, RIX, IMIX generally reflect the skin condition and, thus, depend on the healing stage, general state and the patient's condition. In terms of the determined indices, IMIX seems the most useful in effect of collagen application on wound healing evaluation.

Even though the Ollmar parameters were originally derived for the open-end coaxial probe, they can be used also in the case of 4-electrodes system. In fact, the Ollmar indices possess a diagnostic value and distinguishes different conditions of the skin: ulcerated and non-ulcerated. The use of disposable electrodes in the clinical practice is essential for the patient's safety, therefore the proposed method applying the Ollmar indices as a diagnostic tool would constitute an interesting alternative in pathological skin condition evaluation.

Although the Ollmar indices bear out the effect of collagen on ulcerated skin, its clarification needs further research supported by other methods.

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