Contents lists available at ScienceDirect



Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc



Galvanic apparent internal impedance: An intrinsic tissue property

Alex Golberg^a, Haim D. Rabinowitch^{a,b,*}, Boris Rubinsky^a

^a Center for Bioengineering in the Service of Humanity and Society, School of Computer Science and Engineering, The Hebrew University of Jerusalem, Jerusalem, Israel ^b Robert H. Smith Faculty of Agriculture, Food and Environment, Robert H. Smith Institute of Plant Science and Genetics in Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel

ARTICLE INFO

Article history: Received 15 August 2009 Available online 26 August 2009

Keywords: Galvanic cell Tissue Diagnostics Impedance Salt bridge

ABSTRACT

Using basic galvanic cell principles, the ability of tissues to generate electrical current through electrolysis was characterized. Studying Zn/Cu electrolysis in animal organs revealed a fundamental and measurable tissue-specific property – the galvanic apparent internal impedance (GAII), that is most likely related to the salt bridge function of tissues delineated by electrodes. Further to the fundamental knowledge acquired, GAII enables a new diagnostic method to distinguish between tissue types and to determine their health status without a need for expensive calibration, as often required when external power source is used. We demonstrated the GAII sensitivity in detecting tissue ablation with microwave heating or irreversible electroporation. The results open the way for a novel, inexpensive self-powered tissue diagnostic system for a wide range of applications such as minimally invasive tissue health status, ischemia, hydration, real time intra-operative control of minimally invasive surgery, medical imaging, virtual biopsy and many others.

© 2009 Elsevier Inc. All rights reserved.

Introduction

Electrolysis in living tissues has been reported by Galvani [1], already in the 18th century. Shortly thereafter the electrolytic battery made of two electrodes connected by a salt bridge was described by Volta [2]. Modern developments in bioengineering, micro-robotics, implantable devices and microsensors have raised interest in self-powered sources of electricity from implanted electrolytic batteries [3,4]. In the current work, we have undertaken a fundamental study on electrical current generation from animal tissues through electrolysis. The study was performed in vitro, with electrochemical cells made of selected tissues tightly clutched between flat Zn and Cu electrodes. Additionally, galvanic cells were incorporated into a circuit with a variable exterior resistance (R_{ext}) (Fig. 1). Each cell was discharged over a variable external resistance and the voltage generated was measured by a voltmeter (NI cDAQ-9172, NI 9219 plug, National Instruments Ltd., Austin, TX, USA) connected in parallel with the variable resistors across the cell electrodes, and controlled by custom written software (Labview, version 8.4 National Instruments Ltd., Austin, TX, USA).

Materials and methods

Biological samples and electrical measurements. Male Sprague-Dawley rats' internal organs were used throughout. We measured and compared the GAII of freshly harvested liver, lungs and heart, from: (a) untreated – control; (b) irreversibly electroporated; (c) microwaved tissues. For electrolysis, the analyzed tissue was sandwiched between Zn and Cu (Mini Science Inc. NJ, USA) flat parallel electrodes (Fig. 1). For electroporation, tissues were sandwiched between two Al electrodes connected to an electroporator power supply (BTX 830, Harvard Apparatus, Holliston, MA, USA). Ten unipolar, 100 µs long, 2000 V/cm rectangular electrical pulses were delivered at 5 Hz to induce irreversible electroporation without thermal effects [7]. These tissues represent a solid medium with impaired membranes but with no damage to other organic components [7]. For microwaving, tissues were submerged in a 4.9 g/L physiological solution and treated with microwave power of 810 W for 5 min (Crystal WP900AP23 microwave oven; Crystal Machinery Ltd., China), thus causing inactivation of all organic compounds and irreversible damages. Statistical analyses were done using Microsoft Office Excel 2007 external package, and Student *t* test with unequal variances and reported one tail *p*-value.

Animal procedures. Male Sprague–Dawley rats (250–300 g) (Harlan Laboratories, Jerusalem, Israel) received humane care from a properly trained professional. All procedures complied with the National Institute of Health Guide for the care and use of Laboratory Animals, and were approved by the Institutional Animal Care

^{*} Corresponding author. Address: Robert H. Smith Faculty of Agriculture, Food and Environment, Robert H. Smith Institute of Plant Science and Genetics in Agriculture, The Hebrew University of Jerusalem, P.O. Box 12, 76 100 Rehovot, Israel. Fax: +972 8 9462384.

E-mail address: Rabin@agri.huji.ac.il (H.D. Rabinowitch).

⁰⁰⁰⁶⁻²⁹¹X/\$ - see front matter \odot 2009 Elsevier Inc. All rights reserved. doi:10.1016/j.bbrc.2009.08.119



Fig. 1. Architecture of the tissue electrolytic galvanic cell and the measuring system.

and Use Committee of the Hebrew University. Rats were euthanized using ketamine-xylasine overdose and carbon dioxide.

Eight hearts, 10 lung lobes, and 5 livers were harvested from 9 rats. To minimize animal use, freshly harvested organs were obtained from animals used for other studies. The freshly harvested organs were immediately placed in saline and heparin solution at 10 °C for less than 1 h of each individual sacrifice, prior to use in a specific experiment. The order of treatments followed the order of sacrification. The order of actual measurements, however, for the different organs was at random.

Results and discussions

Systematic electrochemical cell analyses were performed in vitro on freshly harvested heart, liver or lung from Sprague– Dawley rats. Fig. 2A shows the voltage produced by the various galvanic cells as a function of the external resistance values across the electrodes. The current passing through the tissue and other properties were calculated from the external resistance and the voltage, using Ohm's law.

The values in Fig. 2B were calculated from the data presented in Fig. 2A, thus showing the current density generated during the discharge from those galvanic cells as a function of the voltage measured between the electrodes and GAII. It is evident that the current density–voltage relationship differs with tissue type. Our measurements were made for cell constants of 4.53 ± 0.52 ,

22.78 ± 2.27 and 32.40 ± 5.71 cm for heart, lung and liver, respectively. [Cell constant, K_{cell} (cm), is defined as the active surface area of an electrode over distance between electrodes.]

Fig. 2B shows that the open circuit voltage (OCV) or electromotive force (emf) of the galvanic cells at zero current is about 0.76 V, a potential consistent with the electrolytic value of Zn in relation to a hydrogen electrode [5]. Actual OCV values ranged between 0.70 and 0.76 V, probably due to electrode's passivation over the course of experiments. The results suggest that oxidation of the Zn electrode occurs and that hydrogen is being reduced at the Cu electrode [5], as depicted in Eq. (1).

$$\begin{array}{lll} & \text{On } Zn: & Zn \to Zn^{++} + 2e^- & E^0 = 0.76 \ V \\ & \text{On } Cu: & 2H^+ + 2e^- \to H_2 & E^0 = 0 \ V \\ & & : Zn + 2H^+ \to Zn^{++} + H_2 & \Delta E^0 = 0.76 \ V \end{array}$$

The voltage at zero current is a fundamental electrochemical property of the electrodes. In this first study we have not tried to optimize the electrolytic reaction at the electrodes, neither through the choice of materials, surface areas and texture nor by the use of various catalysts. Optimization of the electrolytic reactions could be used to determine the expected OCV values.

The Zn/Cu tissue galvanic cell primary mechanism of current production is through electrolysis, where the tissues function as a typical salt bridge between the two electrodes. The data in Fig. 2 on the relationship between current density and voltages suggests that such a cell can be modeled using standard galvanic cell characterization techniques [6], and that they can generate voltage with open circuit value of 0.76 V in series with a galvanic apparent internal resistance of the salt bridge, R_{app} . The nature of the salt bridge seems to dominate the performance of the tissue electrolytic cell (Fig. 2B). When an exterior resistance R_{ext} closes the circuit, (Fig. 1), the value of the R_{app} can be estimated from current measurements, I_d (Eq. (2)), where the galvanic internal (impedance) resistivity (GAIR), incorporates the cell geometry [6].

$$\frac{1}{I_{d}} = \frac{R_{ext}}{OCV} + \frac{R_{app}}{OCV}$$

$$GAIR = R_{app}K_{cell}(\Omega cm)$$
(2)

Plotting the external resistance against I_d (Fig. 3A) exhibits a highly linear relationship between the two parameters, thus supporting our hypothesis that the tissue galvanic cell reacts as an ohmic resistance over a wide range of external loads. The results were used with Eq. (2) to evaluate the GAIR of the different tissues (Fig. 3B).



Fig. 2. Performance of Zn/Cu galvanic cells with freshly harvested liver, lung or heart tissues serving as electrolytic salt bridge. (A) Voltage produced by the electrochemical cells as a function of external resistance. $K_{cell} = 4.53 \pm 0.52$, 22.78 ± 2.27 and 32.40 ± 5.71 cm for heart, lung and liver, respectively. (B) Current produced by the cells as a function of applied voltage error bars (vertical) – one standard deviation; n = 5 for liver, 10 for lung and 8 for heart; Current error bars (horizontal) were calculated from the voltage errors. Standard deviations for current were thus calculated from the measurements obtained for voltage.



Fig. 3. GAIR properties of heart, lung and liver tissues. (A) Characteristic behavior of a galvanic cell with freshly harvested heart, lung and liver tissues serving as a salt bridge. $K_{cell} = 32.40 \pm 5.71$ cm. (B) Calculated GAIR for: heart, lung and liver. (Error bars – one standard deviation, *n* is the number of replications).

Fig. 3B shows that GAIR is tissue specific. We conclude that GAIR is a fundamental distinguishable property of tissues, being $11 \pm 2 \text{ k}\Omega \text{ cm}$, $29 \pm 4 \text{ k}\Omega \text{ cm}$ and $37 \pm 7 \text{ k}\Omega \text{ cm}$, for heart, lung and liver, respectively. Calculated by the Student's method for unequal variances, the one tail *p*-values for the difference between liver and lung is $p < 3 \times 10^{-2}$, liver and heart is $p < 3 \times 10^{-4}$ and lung and heart $p < 8 \times 10^{-9}$, thus implying that GAIR is a reliable measurement for distinguishing between tissue types.

Extrapolations from the above results open the way for a number of applications. Hence, GAII can be used during biopsy as a simple minimally invasive means to distinguish between malignant and non-malignant tissues within a specific organ; for detection of ischemia progression; in micro-robotics for pinpointing the micro-robot location; for the evaluation of the state of health of the diagnosed tissue e.g. [7,8], and more.

To assess the GAII potential in tissue diagnostics, we addressed the issue of minimally invasive surgery with irreversible electroporation [9] or heating (RF or microwave) [10] which currently lacks means to determine the fate of the treated tissue, to ascertain whether it has been destroyed by the treatment or not. This lack of diagnostic means constitutes a substantial impediment in the treatment of cancer. To this end we studied the effect of tissue ablation on GAIR in fresh liver, where minimally invasive surgery is commonly used for treatment of cancer. We compared the GAIR in freshly harvested liver tissues: (a) without treatment – control; (b) following irreversible electroporation (IRE); (c) following microwave heating.

GAIR value for untreated liver is $37 \pm 7 \text{ k}\Omega \text{ cm}$; for electroporated liver $22 \pm 6 \text{ k}\Omega \text{ cm} (p < 6 \times 10^{-3})$; and for microwaved liver $19 \pm 3 \text{ k}\Omega \text{ cm} (p < 1 \times 10^{-3})$ (Fig. 4). These results show that GAIR can be used for differentiation between untreated and treated tissues. The significant decrease in the apparent internal resistivity in the treated tissues is most probably a consequent of the damage caused to the membranes and cell death, respectively. This further suggests that ionic diffusivity through the salt bridge between electrodes is the *raison d'etre* for this phenomenon, as effective diffusivity is maintained when cell membranes remain intact. Possible explanation are the tortuosity of the extracellular space and the relatively low concentration of electrolytes per unit volume when the fluids inside the cells are not actively participating in the ionic transport.

In summary, our novel finding that tissues' GAII specifically characterizes tissue types and their status has many interesting aspects. From a fundamental research point of view we propose that GAII is related both to the specific salt bridge behavior of a given tissue and to the transport of the electrochemical species between electrodes. This has to be further confirmed through fundamental studies by correlating electrochemical species transport during electrolysis and GAII. Proven correct, it opens the way for new techniques and methods to study and to better understand electrical charged species transport in tissues and their electrolytic reaction.

The technology can be applied with no external calibrated power source, and thus used *in vivo* anywhere inside the body. Indeed, the value of any technique based on the galvanic apparent internal impedance property is its application without the need for external calibrated of the power source.

Fundamental in vitro and in vivo studies of tissues' GAII should generate a database with a significant consequence on basic knowledge, as well as for practical applications. Similar studies for the determination of the tissues' electrical properties were made using external power sources [11,12]. To the best of our knowledge no reports are available on the GAII of the studied tissues. On the basis of the results presented in this study, we propose that this property can be characterized and then employed for all applications currently using tissue impedance based on conventional external electrical power sources. The determination of the galvanic apparent internal impedance only requires a single pair of electrodes with specific chemical potentials which can be predetermined by a precise control of the electrolytic reaction. Therefore, unlike conventional electrical tissue properties based applications; it can be employed without expensive power field generators.

We hereby propose that the measurings of the property identified in this study may emerge as an efficient and accurate way for



Fig. 4. Values of liver GAIR as a function of IRE or microwave treatments. The following electroporation protocol was applied: 10 consecutive pulses of 2000 V/ cm, $100 \,\mu\text{s}$ long at 1 Hz. Microwave treated tissues were exposed to $810 \,\text{W}$ microwave power for 5 min in saline. Error bars – one standard deviation, n = number of replications.

tissue diagnostics and to control tissue treatment/ablation techniques in real time. By combining the new capabilities afforded by its identification with the advantages of power generator-free diagnostic devices, the proposed method may help developing low cost accurate medical/food technology devices with unprecedented opportunities for the diagnostics, real time monitoring, remote control and modeling of tissue processes.

References

- L. Galvani, De viribus electricitatis in motu musculari commentarius, Bon. Sci. Art. Inst. Acad. Comm. 7 (1791) 363–418.
- [2] A. Volta, On the electricity excited by the mere contact of conducting substances of different species, Philos. Trans. Roy. Soc. 90 (1800) 403–431.
- [3] J.R. Rao, G. Richter, Implantable bio-electrochemical power sources, Naturwissenschaften 61 (1974) 200–206.
- [4] O.Z. Roy, R.W. Wehnert, Improvements in biogalvanic energy sources, Med. Biol. Eng. Comput. 12 (1974) 50–56.

- [5] P. Vanysek, Electrochemical series, in: D.R. Lide (Ed.), Handbook of Chemistry and Physics, 89th ed., Taylot & Francis Inc., 2007.
- [6] H.A. Kiehne, Battery Technology Hand Book, second ed., Marcel Dekker Inc., NY, 2003.
- [7] A. Ivorra, B. Rubinsky, In vivo electrical impedance measurements during and after electroporation of rat liver, Bioelectrochemistry 70 (2006) 287–295.
- [8] S. Laufer, B. Rubinsky, Tissue characterization with an electrical spectroscopy SVM classifier, IEEE Trans. Biomed. Eng. 56 (2009) 525–528.
- [9] B. Rubinsky, Irreversible electroporation in medicine, Technol. Cancer Res. Treat. 6 (2007) 255–260.
- [10] M. Williams, M. Knaut, D. Bérubé, M.C. Oz, Application of microwave energy in cardiac tissue ablation: from in vitro analyses to clinical use, Ann. Thorac. Surg. 74 (2002) 1500–1505.
- [11] C. Gabriel, S. Gabriel, E. Corthout, The dielectric properties of biological tissues. I. Literature survey, Phys. Med. Biol. 41 (1996) 2231–2249.
- [12] S. Gabriel, R.W. Lau, C. Gabriel, The dielectric properties of biological tissues: III. Parametric models for the dielectric spectrum of tissues, Phys. Med. Biol. 41 (1996) 2271–2293.