

Bioimpedance dispersion width as a parameter to monitor living tissues

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Received 26 August 2004, accepted for publication 25 October 2004

Published DD MMM 2004

Online at stacks.iop.org/PM/26/1

Abstract

In the case of living tissues, the spectral width of the electrical bioimpedance dispersions (closely related with the α parameter in the Cole equation) evolves during the ischemic periods. This parameter is often ignored in favor of other bioimpedance parameters such as the central frequency or the resistivity at low frequencies. The object of this paper is to analyze the significance of this parameter through computer simulations (in the α and β dispersion regions) and to demonstrate its practical importance through experimental studies performed in rat kidneys during cold preservation. The simulations indicate that the dispersion width could be determined by the morphology of the extra-cellular spaces. The experimental studies show that it is the unique parameter able to detect certain conditions such as a warm ischemia period prior to cold preservation or the effect of a drug (Swinholide A) able to disrupt the cytoskeleton. The main conclusion is that, thanks to the α parameter in the Cole equation, the bioimpedance is not only useful to monitor the intra/extra-cellular volume unbalances or the inter-cellular junctions resistance but also to detect tissue structural alterations.

Keywords: dispersion width, cytoskeleton, monitoring, morphology, Cole, bioimpedance

(Some figures in this article are in colour only in the electronic version)

1. Introduction

One of the first successful electrical models for the electrical passive properties of living tissues was introduced by Fricke and Morse in 1925 (Cole 1972). It consists of a resistance for the

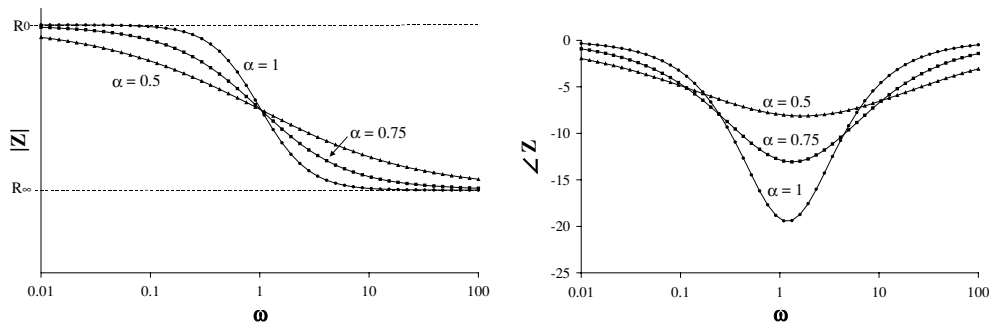


Figure 1. Impedance magnitude and phase angle from Cole equation for three values of α .

extra-cellular electrolytic medium (R_e) in parallel with the series combination of a resistance for the intra-cellular electrolytic medium (R_i) and a capacitance for the cell membrane (C_m). This model represents one Debye type relaxation process with a single characteristic time constant and, in the frequency domain, corresponds to a single dispersion, that is, a transition between two frequency-independent levels (see Grimnes and Martinsen (2000)) for more details about these concepts).

The Fricke–Morse model has been extensively used and, even today, some authors make use of it (Haemmerich *et al* 2002, Konishi *et al* 1995) because of its simplicity and because it is able to describe qualitatively the observed main dispersion in the β dispersion region as defined by Schwan (1957). However, since the first studies, it was observed that this capacitive model was not accurate enough to fit the experimental results in cell suspensions or living tissues studies (Cole 1972). When the impedance values measured at multiple frequencies are represented in a Bode plot, it is observed that the behavior of the capacitive model is **stepper** that the results from the actual bioimpedance characterization. Moreover, in the Nyquist plot (imaginary part versus real part) both, the capacitive model and the experimental results, produce a semicircle but, in the case of the actual data the center is not on the real axis. This phenomenon is usually described as ‘depressed semicircles’.

Cole (1940) introduced the first mathematical expression able to describe the ‘depressed semicircles’ found experimentally. It is known as the Cole equation (here expressed as in Grimnes and Martinsen (2000)):

$$\mathbf{Z} = R_\infty + \frac{\Delta R}{1 + (j\omega\tau)^\alpha}, \quad \Delta R = R_0 - R_\infty \quad (1)$$

where \mathbf{Z} is the impedance value at frequency ω , j is the complex number $(-1)^{1/2}$, R_∞ is the impedance at infinite frequency, R_0 is the impedance at zero frequency, τ is the characteristic time constant and α is a dimensionless parameter with a value between 0 and 1. Figure 1 shows the impedance magnitude and phase from the Cole equation for different values of α . Note that α is closely related with the spectral width of the dispersion; the minimum spectral width corresponds to $\alpha = 1$ and the dispersion is broadened as α tends to lower values. For that reason, here ‘dispersion width’ and α are used indistinctively. The α value can also be regarded as a parameter denoting the derivation from Fricke–Morse model. That is, the Cole equation (1) with $\alpha = 1$ is equal to the Fricke–Morse model.

Living tissues usually exhibit one or two dispersions in the α and β dispersion regions (from 1 Hz to some tens of MHz) that can be well approached by series combinations of (1). In those cases, the α values are around 0.8 (note that α in the Cole equation has nothing to do with the ‘ α dispersion region’). The physical phenomenon underlying each dispersion is generally

See endnote 1

understood. For instance, the large dielectric dispersion appearing in the β dispersion region is considered to be associated with the dielectric properties of the cell membranes and their interactions with the extra- and intra-cellular electrolytes. However, the physical meaning of α is not clearly understood. That is, there is no agreement on the cause of $\alpha < 1$. Most authors suggest that it is caused by the heterogeneity of cell sizes and shapes in a living tissue (Ackmann and Seitz 1984, Foster and Schwan 1989). This explanation is based on the fact that a random distribution of relaxation times (time constants) with a certain probability density function will indeed produce an impedance spectrogram compatible with (1). Therefore, it seems reasonable to think that the heterogeneity of an actual tissue could produce such a random distribution. However, to produce α values around 0.8 a very broad distribution of relaxation times would be necessary that cannot be related to the actual heterogeneity of tissues in terms of cell sizes and shapes. Hence, the presence of mitochondrias and other compartments in the cell has also been suggested as an explanation to the broad distribution of relaxation times (Raicu *et al* 1998), however, it is more generally accepted that those organelles cause differentiable sub-dispersions at high frequencies (Asami *et al* 1996, Foster and Schwan 1989) rather than dispersion broadening. Among the other proposed theories to explain derivations from Fricke–Morse model, the fractal interpretation postulated by Dissado must be mentioned (Dissado 1990). He showed that the hierarchical organization of biological tissues can justify the frequency power law dependency of the dielectric response of animal tissues.

However, none of these theories can explain the fact that homogeneous clusters of cells without any hierarchical organization show the Cole behavior. Moreover, the above theories do not easily explain the fact that α evolves with time under certain circumstances (Osypka and Gersing 1995, Raicu *et al* 2000). Our experiments on cold preservation of rat kidneys (see the experimental study section) show such a behavior and, what it is more significant, the evolution of α is completely independent of the rest of bioimpedance parameters. It seems that α follows some sort of induced damage that has nothing to do with the cellular edema. We do believe that α is closely related with the morphology of the extra-cellular spaces and this paper is intended to show evidences in this sense:

- (1) By performing computer simulations, here it is shown that the morphology of the extra-cellular spaces will indeed determine the value of α .
- (2) Since the cell morphology depends on the cytoskeleton, we have decided to test the effect of a drug (Swinholide A) that alters the cytoskeleton because it severs actin filaments and sequesters actin dimers (Bubb and Spector 1998).

It must be noted that there are some previous works that indicate that deviations from the Cole model are related somehow to cell aggregation or clusterization. For instance, it has been shown that α decreases when blood hematocrit increases (Fomekong *et al* 1998) or that dispersion broadening occurs during embryogenesis (Asami and Irimajiri 2000). In this sense, the present paper can be considered a reinforcement of that interpretation.

2. Computer simulations

There exist some attempts to relate the tissue structures with the bioimpedance measurements by using analytical models linked with some physical properties of cells and tissues (Raicu *et al* 1998). These analytical models can work properly in some cases such as cell suspensions, however, their applicability is limited when complex structures, such as living tissues, are considered. In those cases, it may be advisable to use computer models and simulations. It is possible to find some examples of computer simulations of electrical bioimpedance at cellular level (Jones *et al* 2003, Walker *et al* 2000). Most of those simulations are based on

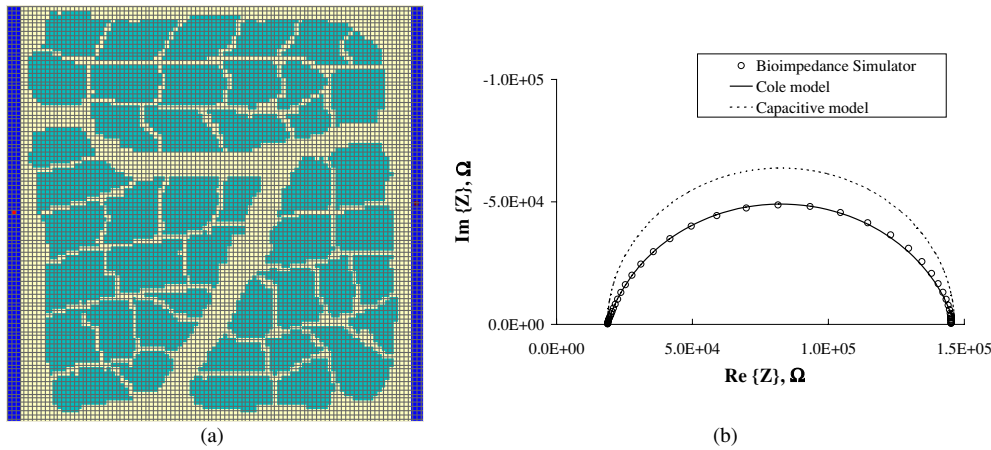


Figure 2. (a) Simulated structure resembling an actual tissue cut (100×100 squares, slice thickness = $50 \mu\text{m}$, pixel size = $2 \mu\text{m}$, membrane capacitance = $1 \mu\text{F cm}^2$, plasm resistivity = cytoplasm resistivity = $100 \Omega \text{ cm}$, electrode resistivity = $0.001 \Omega \text{ cm}$). (b) Nyquist plot of the simulated tissue impedance.

finite element analysis and that implies the use of software tools that require a high degree of expertise because of its complexity.

Here, a custom developed simulator has been employed in order to simplify the simulation of the electrical impedance of living tissues. This software, the ‘bioimpedance simulator’, generates SPICE input files, also referred to as netlists. SPICE stands for ‘simulation program with integrated circuit emphasis’ and it was originally conceived more than 30 years ago at the University of California at Berkeley to simulate and predict the behavior of electronic circuits (Al-Hashimi 1995). The ‘bioimpedance simulator’ including its help documentation and its Visual Basic source files are available at <http://www.cnm.es/~mtrans/BioZsim/>.

The ‘bioimpedance simulator’ generates a netlist that represents a flat section, or more precisely a slice, of living tissue. Basically, it consists of a bi-dimensional mesh of passive electric components that depend on some numerical parameters, such as the plasm and cytoplasm resistivities, and a bi-dimensional map drawn by the user. Each square pixel of the map is transformed into a set of passive circuit components (resistances and capacitances) interconnected between them and the components of the adjacent pixels. The electrode, plasm (extra-cellular medium) and cytoplasm elements are modeled as pure resistive media. At any plasm–cytoplasm interface, the presence of the cell membrane is assumed and it is modeled as a capacitance in parallel with a resistance. Of course, such elements do not produce Cole responses by themselves. Precisely the idea behind the current simulations was to demonstrate that, when these elements are combined because of the complex structure of living tissues, the overall response can differ from the simple Fricke–Morse model and approach to the Cole model.

2.1. Generic tissue

The electrical bioimpedance of a structure resembling a generic tissue cut (figure 2(a)) was obtained by the ‘bioimpedance simulator’. It represents three cell clusters separated by large extra-cellular spaces (vessels). The results (figure 2(b)) not only differ from the capacitive behavior (Fricke–Morse model) but also match the Cole response found in actual measurements. By using a commercial software (ZView, Scribner Associates, Inc), the

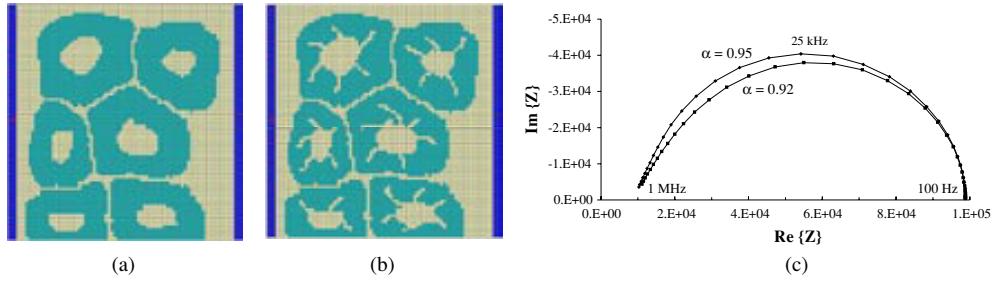


Figure 3. Simulated cross sections of renal tubules (a, b) and the obtained responses (c). (Simulation parameters: 80×80 squares, slice thickness = $50 \mu\text{m}$, pixel size = $2 \mu\text{m}$, membrane capacitance = $1 \mu\text{F cm}^2$, plasma resistivity = cytoplasm resistivity = $100 \Omega \text{ cm}$, electrode resistivity = $0.001 \Omega \text{ cm}$).

following values are obtained for the Cole equation (1): $R = 18.7 \text{ k}\Omega$, $\Delta R = 127.6 \text{ k}\Omega$, $\alpha = 0.835$ and $\tau = 2.88 \mu\text{s}$. It must be noted that the Cole behavior disappears (α tends to 1) if the extra-cellular spaces are widened.

2.2. Kidney tissue

In order to link the simulations to the experimental study described below, a slab of kidney tissue was also modeled. Figure 3(a) shows the simulated cross section of renal tubules. Observe that each tubule has been simulated as a single cell with a large plasma vesicle inside it. Of course, that is not realistic since each tubule is formed by multiple cells but, considering the tight packaging of these cells and the presence of inter-cellular junctions (gap junctions), it is a reasonable simplification. In figure 3(c), the obtained impedance locus for this case has been represented; the response can be nicely modeled by a Cole response with $\alpha = 0.95$. In the case that some degree of imperfections are added to the structure (figure 3(b)), the impedance response significantly differs from the original one, specially for the α value (from 0.95 to 0.92). The added imperfections try to mimic the detachment between the tubule cells that has been observed in scanning electron microscopy (SEM) micrographs after preservation when the kidneys were treated with Swinholide A (see the experimental section).

3. Experimental study

In a previous experimental study (Genescà *et al* 2004), we found that the evolution of α during the preservation of rat kidneys was significantly altered in the case that a warm ischemia episode was induced before kidney extirpation and preservation. Two rat kidney groups were studied during 24 h of preservation in University of Wisconsin solution (UW): a control cold ischemia group and another group subjected previously to 45 min of warm ischemia. The time constant and the high frequency resistivity parameters derived from the Cole equation were able to discriminate between both groups at the beginning of the preservation ($\Delta\tau \sim 78\%$, $\Delta R_\infty \sim 36\%$), but these differences tended to converge as preservation time advanced. On the other hand, the α showed increasing significant differences until 24 h of preservation ($\Delta\alpha \sim 15\%$).

One of the conclusions drawn from that study was that during renal preservation α apparently followed the cellular morphological changes caused by cytoskeleton disruption and that are related to ischemia (Kellerman and Bogusky 1992). This fact motivated the study

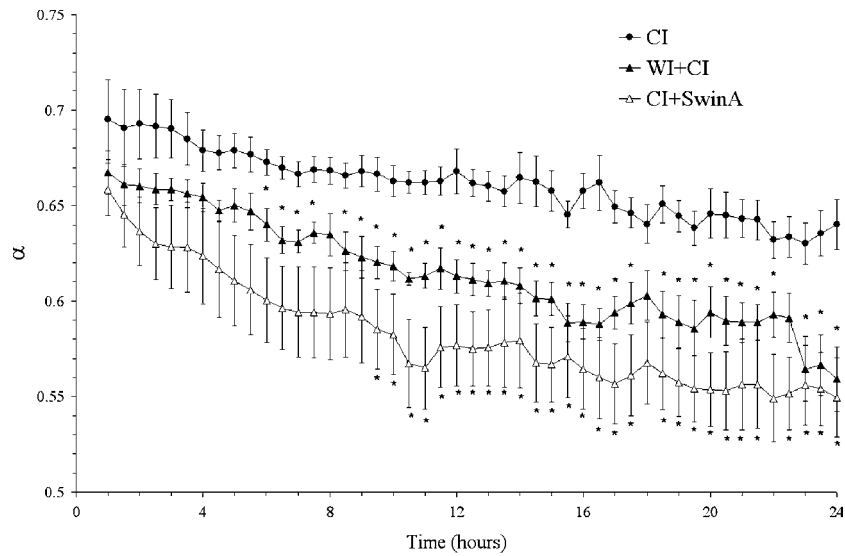


Figure 4. Time evolution of the α parameter from the Cole equation (adimensional) during 24 h of cold preservation. Results are expressed as mean \pm standard error of the mean.

presented here: to add a drug that severely disrupts the cytoskeleton in order to reinforce the hypothesis that α is somehow linked to the cell morphology.

Electrical bioimpedance of rat kidneys was monitored during 24 h of preservation in three groups (four animals per group):

- (1) *Cold ischemia group (CI)*: kidneys were isolated and preserved for 24 h in University of Wisconsin (UW) preservation solution.
- (2) *Warm ischemia group (WI+CI)*: kidneys underwent a prior 45 min of warm ischemia before isolation and cold storage.
- (3) *Swinholide group (CI+SwinA)*: kidneys were treated with Swinholide A (flushed 500 μ l of UW containing 500 nM of SwinA) and followed the same procedure as for those in the CI group.

The study was performed using male Wistar rats weighing between 250 and 300 g. Animals were anesthetized by injection of sodium pentobarbital (30 mg kg⁻¹) and placed in a supine position, keeping body temperature between 36 °C and 37 °C. All procedures were conducted under the supervision of our institution's Research Commission and followed EU guidelines for the handling and care of laboratory animals (see Genescà *et al* (2004)) for further methodological details).

Electrical bioimpedance was measured by using a miniaturized probe that consisted of four platinum electrodes (300 μ m \times 300 μ m) on a needle shape silicon substrate (9 mm insertion length; 600 μ m \times 500 μ m cross section). An analysis of the probe properties and a description of the instrumentation system can be found elsewhere (Ivorra *et al* 2003).

As it can be observed in figure 4, the WI+CI and the CI+SwinA groups produced a similar response for the α parameter: a significant faster decrease than for the case of CI group. The other parameters (R_{∞} , R_0 and τ) were different between three groups at the beginning of the preservation (see Genescà *et al* (2004)) but converged after 24 h. SEM micrographs revealed a lower degree of disruption of neighboring cell junctions in the CI group.

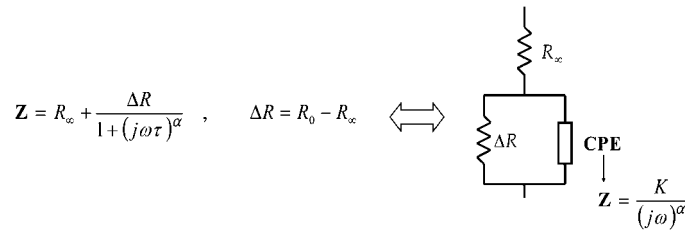


Figure 5. The Cole equation and its equivalent electrical circuit.

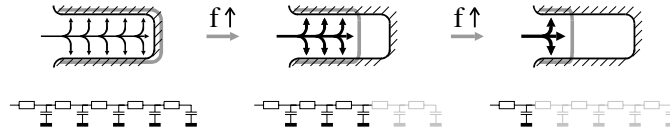


Figure 6. Representation of the electrode pore impedance dependence on frequency. As frequency increases (f), the current tends to flow through the outer areas of the pore and that reduces the effective capacitance and the resistance.

4. Discussion

Up to now, no agreement exists on the physical meaning of the α parameter in the Cole equation for the case of living tissues. Several theories have been proposed but none of them is able to completely explain all the associated phenomena. Probably all the postulated causes do certainly contribute to the α value and the main point would be to identify which one represents the most significant contribution. Here it is proposed that the α value is related with the morphology of the extra-cellular spaces that, of course, depends on the morphology of the cells. That is not necessarily opposite to previous theories; it can be regarded as another interpretation. However, here this explanation is supported by strong evidences.

The dependence of the α parameter on the morphology of the extra-cellular spaces can be understood intuitively. In the equivalent electrical circuit of the Cole equation (figure 5), the constant phase element (CPE) is depicted (Grimnes and Martinsen 2000). The behavior of this non-physical element is equivalent to a resistance–capacitance series combination whose values depend on the frequency. Such a behavior is also observed in the interface between a solid electrode and an electrolyte (McAdams 1989). In this case, the CPE response has been intensively studied and it has been found that the α value could depend on the morphology and the dimensions of the electrode surface irregularities (De Levie 1965, Liu 1985, Nyikos and Pajkossy 1985). If these irregularities are seen as pores, the dependence of the CPE on the frequency can be explained intuitively (see figure 6): as frequency increases, the electrode area ‘seen’ by the current from the electrolyte is reduced and the capacitance caused by the double layer is reduced; at the same time, the current reduces its penetration into the pore and so does the resistive component. In living tissues, the cell membrane would be equivalent to the double layer of the electrode–electrolyte interface and the narrow extra-cellular spaces would be equivalent to the electrolyte pores. That is, the cell membrane area ‘seen’ by the injected currents into the tissue sample would depend on the frequency and so does the capacitance and the resistance. Of course, that does not explain the Cole equation but helps us to understand that it is possible to obtain a dependence of the resistive and capacitive components on the frequency in the case of living tissues.

The computer simulations presented here show that the Cole behavior can be caused by the structure of the tissues at cell level; there is no need to assume special properties of the cell membrane or to consider the contribution of intra-cellular organelles. Moreover, other performed simulations, not shown here, including the presence of organelles or large cell dimension differences tend to produce double-dispersion responses rather than Cole responses. That is, we have found that in order to obtain impedance responses similar to the actual measurements, the key point is to consider a more or less complex network of extra-cellular narrow spaces in which the cells are embedded.

As it can be observed, the kidney tissue simulations do not fit so accurately the experimental measurements. The initial value of α in the case of the simulation is 0.95 whereas in the experimental results is around 0.7. That is caused by the poor resolution of the simulation and an oversimplification. For instance, the presence of microvilli inside the pore is completely ignored since their diameters would be lower than the simulator resolution. However, the important fact is that the modification of the extra-cellular medium in order to mimic the cell detachment produces a decrease of the simulated α value of the same order of magnitude than the actual α decrease during preservation.

The experimental results reinforce the hypothesis that the α parameter is related with the extra-cellular morphology. The use of Swinholid decreases the α value without inducing large changes in the other Cole parameters (R_∞ , R_0 and τ). That implies that the α parameter evolution in this case is not related with cell edema nor gap junction closure, which are common causes of bioimpedance changes. Therefore, the drug only modifies the α parameter and, since this drug acts mainly on the cells cytoskeleton, it can be concluded that there exists a relation between the cytoskeleton condition and the α parameter. Since there is no reason to think that the cytoskeleton by itself contributes significantly to the bioimpedance measurements, it is reasonable to think that there exists an indirect relation between it and the α parameter. We do believe that this relation is caused by the direct relation between the cytoskeleton and the cell morphology (Molitoris *et al* 1997).

5. Conclusions

It is not possible to conclude definitely that the α parameter in the Cole equation is related with the extra-cellular space morphology, but, some evidences have been presented that reinforce this hypothesis.

Although the α parameter is often ignored in favor of other bioimpedance parameters, here its potential application to the characterization of living tissues has been demonstrated. Therefore, its measurement is strongly recommended since it provides independent information from other parameters such as R_0 and τ . However, it must also be mentioned that the relatively small changes of the α parameter can constrain its applicability unless a fine measurement set-up is employed.

Acknowledgments

This study was supported by the European Commission through projects ESPPRIT-LTR-23485, IST-1999-13047 and QLK6-CT-2000-00064 and by the Spanish government through projects TIC98-1634-CE, TIC2000-2486-CE, FISS 01/1691 and SAF 2000-3090-CE, SAF 2003-04225 CE.

References

- Ackmann J J and Seitz M A 1984 Methods of complex impedance measurements in biologic tissue *Crit. Rev. Biomed. Eng.* **11** 281–311
- Al-Hashimi B 1995 *The Art of Simulation Using PSpice: Analog and Digital* (Boca Raton, FL: CRC Press)
- Asami K and Irimajiri A 2000 Dielectrospectroscopic monitoring of early embryogenesis in single frog embryos *Phys. Med. Biol.* **45** 3285–97
- Asami K, Yonezawa T, Wakamatsu H and Koyanagi N 1996 Dielectric spectroscopy of biological cells *Bioelectrochem. Bioenerg.* **40** 141–5
- Bubb M R and Spector I 1998 Use of the F-actin-binding drugs, misakinolide A and swinholide A *Methods Enzymol.* **298** 26–32
- Cole K S 1940 Permiability and impermeability of cell membranes for ions *Sympos. Quant. Biol.* pp 110–22
- Cole K S 1972 *Membranes, Ions and Impulses* (Berkeley: University of California Press)
- De Levie R 1965 The influence of surface roughness of solid electrodes on electrochemical measurements *Electrochim. Acta* **10** 113–30
- Dissado L A 1990 A fractal interpretation of the dielectric response of animal tissues *Phys. Med. Biol.* **35** 1487–503
- Fomekong R D, Pliquet U and Pliquet F 1998 Passive electrical properties of RBC suspensions: changes due to distribution of relaxation times in dependence on the cell volume fraction and medium conductivity *Bioelectrochem. Bioenerg.* **47** 81–8
- Foster K R and Schwan H P 1989 Dielectric properties of tissues and biological materials: a critical review *CRC Crit. Rev. Biomed. Eng.* **17** 25–104
- Genescà M, Ivorra A, Sola A, Palacios L, Villa R and Hotter G 2004 Electrical bio-impedance monitoring of rat kidneys during cold preservation by employing a silicon probe *Proc. from the 12th Int. Conf. on Electrical Bio-Impedance (ICEBI) (Gdansk)* pp 127–30
- Grimnes S and Martinsen Ø G 2000 *Bioimpedance and Bioelectricity Basics* (London: Academic)
- Haemmerich D, Ozkan O R, Tsai J Z, Staelin S T, Tungjitkusolmun S, Mahvi D M and Webster J G 2002 Changes in electrical resistivity of swine liver after occlusion and postmortem *Med. Biol. Eng. Comput.* **40** 29–33
- Ivorra A, Gómez R, Noguera N, Villa R, Sola A, Palacios L, Hotter G and Aguiló J 2003 Minimally invasive silicon probe for electrical impedance measurements in small animals *Biosensors Bioelectron.* **19** 391–9
- Jones D M, Smallwood R H, Hose D R, Brown B H and Walker D C 2003 Modelling of epithelial tissue impedance measured three different designs of probe *Physiol. Meas.* **24** 605–23
- Kellerman P S and Bogusky R T 1992 Microfilament disruption occurs very early in ischemic proximal tubule cell injury *Kidney Int.* **42** 896–902
- Konishi Y, Morimoto T, Kinouchi Y, Iritani T and Monden Y 1995 Electrical properties of extracted rat liver tissue *Res. Exp. Med.* **195** 183–92
- Liu S H 1985 Fractal model for the ac response of a rough interface *Phys. Rev. Lett.* **55** 529–32
- McAdams E T 1989 Effect of surface topography on the electrode–electrolyte interface impedance, 1. The high frequency ($f > 1$ Hz), small signal, interface impedance—a review *Surf. Topogr.* **2** 107–22
- Molitoris B A, Leiser J and Wagner M C 1997 Role of the actin cytoskeleton in ischemia-induced cell injury and repair *Pediatr. Nephrol.* **11** 761–7
- Nyikos L and Pajkossy T 1985 Fractal dimension and fractional power frequency-dependent impedance of blocking electrodes *Electrochim. Acta* **30** 1533–40
- Ospyka M and Gersing E 1995 Tissue impedance spectra and the appropriate frequencies for EIT *Physiol. Meas.* **16** A49–55
- Raicu V, Saibara T, Enzan H and Irimajiri A 1998 Dielectric properties of rat liver *in vivo*: analysis by modeling hepatocytes in the tissue architecture *Bioelectrochem. Bioenerg.* **47** 333–42
- Raicu V, Saibara T and Irimajiri A 2000 Multifrequency method for dielectric monitoring of cold-preserved organs *Phys. Med. Biol.* **45** 1397–407
- Schwan H P 1957 Electrical properties of tissue and cell suspensions *Advances in Biological and Medical Physics* vol 5 ed J H Lawrence and C A Tobias (New York: Academic)
- Walker D C, Brown B H, Hose D R and Smallwood R H 2000 Modelling the electrical impedivity of normal and premalignant cervical tissue *Electron. Lett.* **36** 1603–4

See endnote 2

Endnotes

- (1) Author: The meaning of 'stepper that' is unclear. Please check.
- (2) Author: Please check whether Cole (1940) is OK as typeset.
- (3) Author: Please be aware that the colour figures in this article will appear in colour only in the Web version. If you require colour in the printed journal and have not previously arranged it, please contact the Production Editor now.