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Design and implementation of a novel superfusion system for *ex vivo* characterization of neural tissue by dielectric spectroscopy (DS)

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Abstract
Dielectric spectroscopy is a widely utilized electrophysiological characterization method. The obtained dielectric spectra and derived properties have the potential of providing significant information regarding changes in the physiological state of a biological system. However, since many of the dielectric properties are obtained *in vitro* from excised tissue far removed from physiological conditions, the value of the information obtained may be diminished. In this paper, we introduce a superfusion system that is designed to produce *ex vivo* dielectric spectroscopy measurements by providing the living tissue with a continuous and ample supply of nutrients and oxygen while removing metabolites and other waste. This superfusion system provides the convenience of *in vitro* measurement while concurrently producing results that can be more closely correlated with actual physiological changes in the biological system.

Keywords: dielectric spectroscopy, superfusion, neural tissue, *ex vivo*

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

1. Introduction

The field of electrophysiology encompasses the study of a biological system's intrinsic electrical properties. Several techniques, including patch clamping, imaging with voltage-sensitive dyes, and dielectric/impedance spectroscopy, are currently utilized by researchers

to quantify the electrophysiological properties of a system. These methods are useful for the study of disease states because these states are often characterized by alterations in electrophysiological properties. For example, neural pathologies such as neurodegeneration (Hartley *et al* 1999, Ye *et al* 2004), cerebral concussion (Gennarelli 1986), blepharospasm, and oromandibular dystonia (Berardelli *et al* 1985) are all characterized by electrophysiological changes at the cellular and tissue levels. Among the various electrophysiological techniques, dielectric spectroscopy has emerged as a powerful method for characterizing biological systems because it is non-invasive and label free (Schwan 1957, Gheorghiu 1993, Rigaud *et al* 1996, Prodan *et al* 2004, Dean *et al* 2008). It has been used to characterize biological systems over a large range of sizes—from peptides within supported membranes (Asami *et al* 2002) to whole tissue and organ samples (Schwan 1957, Raicu *et al* 2000), and is a useful method for the study of cellular death (McRae *et al* 1999, Patel and Markx 2008).

The dielectric properties of a biological system are closely related to the overall health of the system (Gheorghiu 1996), making it a potentially valuable method for measuring the extent of physiological changes associated with disease states. Citing this relationship, there is a long history of scientists and engineers investigating the electrical properties that govern the actions of biological systems. In the latter part of the 19th century, researchers began to notice that the resistance of biological tissue decreases as the frequency of the applied signal increases (Nightingale 1958). While studying the resistance of frog's skin, Gildemesiter (1912) showed that the impedance of tissue contains a reactive component. Utilizing data and theories offered by his predecessors, Cole (1928, 1932) postulated not only that the impedance of each cell membrane varied with frequency but also that it is quantitatively represented by the sum of its real and imaginary components. Furthermore, he theorized that the equivalent electrical circuit of the tissue can be modeled by the series combination of a frequency-dependent capacitance and resistance. Taking advantage of the emerging theories of his contemporaries and predecessors, Herman P Schwan worked in the field of electrical properties of biological materials and led a profound career (Foster 2002). An excellent overview of the chronological development of dielectric properties is offered by McAdams and Jossinet (1995).

More recently, some dielectric spectroscopy measurements have been taken *in vivo*, such as the use of external bioimpedance sensors (Benjamin *et al* 2005, Seoane *et al* 2005) and measurements made during surgery (Beltran *et al* 2006). However, the majority of published data concerning the dielectric properties of tissue comes from excised tissue far removed from physiological conditions (Foster and Schwan 1996). Gabriel *et al* (1996) and Gabriel (2007) have compiled a database of various dielectric properties for nearly every major mammalian organ system while concurrently offering an excellent overview of our current understanding of the dielectric properties of biological tissue. As noted by Foster and Schwan (1996), changes in the dielectric properties observed in these data do not necessarily correspond to changes in important physiological variables within the intact system. Therefore, changes to the electrical properties seen in these tabulated *in vitro* data, may not necessarily correspond to changes observed *in vivo*.

Henry McIlwain was one of the earliest pioneers in brain slice preparations and subsequent efforts to maintain excised tissue viability by superfusion (Collingridge 1995). The earliest superfusion chambers were custom-made apparatuses intended for application-specific measurements of neural function (Yamamoto and McIlwain 1966, White *et al* 1978, Alger and Nicoll 1981). These early superfusion systems proved capable of maintaining neural tissue viable for up to 12 h *in vitro* (Haas *et al* 1979). These same superfusion systems gave way to commercial apparatuses such as the Coleman superfusion bath system (Harvard Apparatus, Holliston, MA). While a wide diversity of superfusion system designs have been described in the literature, no system has been designed specifically for use with dielectric

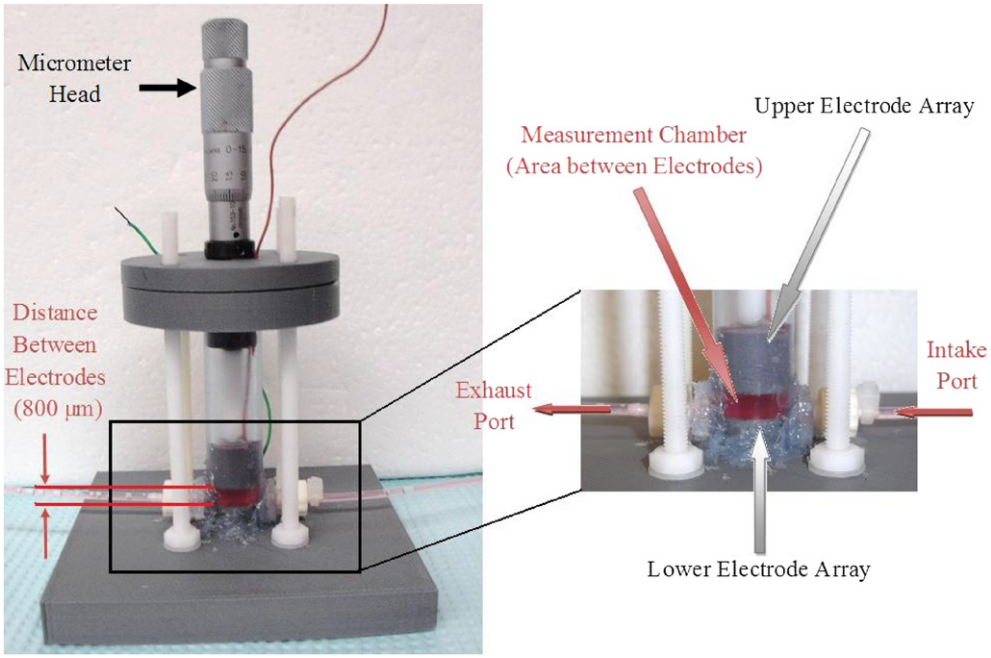

Figure 1. Image of the custom-made parallel plate electrode array designed and built by the authors. The gray members in the image are comprised of ABS thermoplastic and were produced using the rapid prototyping technology. The electrode material is 14 k white gold due to its cathodic (inert) properties as well as its low resistivity. A metric micrometer head precisely controls the separation distance between the electrodes.

spectroscopy. The system presented here is similar to previously described apparatuses in that it provides tissue with oxygen and nutrient-rich extracellular solution. This apparatus differs from other systems, however, in that it allows for continuous superfusion of brain slice preparations while simultaneously performing dielectric spectroscopy.

In this paper, we introduce a novel superfusion system designed to maintain the viability of brain slices in order to increase the correlation between measurements made *in vitro* to the actual dielectric properties of biological tissue *in vivo*. The system increases this correlation by continuously providing a single brain slice with nutrients (i.e. glucose) and oxygen while also allowing for simultaneous *ex vivo* dielectric spectroscopy measurements. While this superfusion system could be in principle applied to any tissue sample, our desire to specifically investigate the dielectric properties of neural tissue necessitated the design of this system. The mammalian nervous system is very metabolically active, with the human brain being responsible for approximately 20% of resting oxygen consumption (Kety 1957).

2. Materials and methods

2.1. Design of the electrode system

The integral component of the superfusion system is the custom designed electrode array as shown in figure 1. This general design has previously been shown to measure accurate values of the complex dielectric permittivity of a variety of materials (Prodan *et al* 2004, Bot and

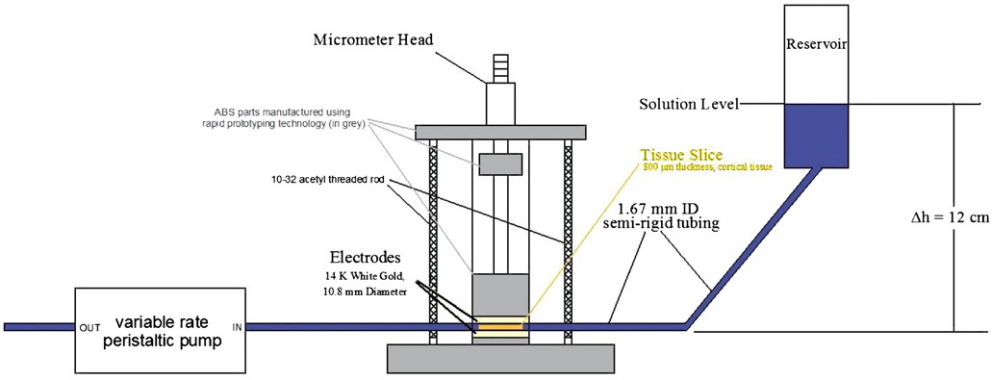

Figure 2. The key aspects of the novel superfusion system are the custom-designed and built gold electrode array, the gravity-fed input component, and the exhaust component, which is moderated by a peristaltic pump. The purpose of the system is to provide nutrient- and oxygen-enriched electrolytic solution to the tissue sample while removing metabolite- and waste-enriched solution. This superfusion system is intended to mimic physiological conditions as closely as possible in the compartment that houses the tissue sample.

Prodan 2009, Prodan and Bot 2009). At the core of this parallel plate electrode array are two 14 k white gold disk electrodes 10.8 mm in diameter. Fourteen karat white gold was chosen for the electrode material with consideration to its low resistivity and cathodic nature. The inert properties of the gold minimize reactions with the strong electrolytic solutions at the electrode–solution interface.

The electrodes were enclosed in a clear polyvinyl chloride (PVC) pipe (ALSCO Industrial Products Inc., Lithia Springs, GA 30122) with an inner diameter of 12 mm and a length of 76 mm. The diameter of the upper and lower electrodes was approximately 1 mm smaller in size than the inner diameter of the PVC tube enclosure. This close fit reduced the error caused by stray capacitance at the edge of the electrodes by constraining the stray electric field line to go through either the PVC tube enclosure or the air. Since the dielectric permittivity of both these materials is much lower than that of the electrolytic solution, the contribution of the stray capacitance on the overall measurement was minimized.

The separation distance between the electrodes was precisely controlled by a metric micrometer head with non-rotating spindle (Mitutoyo Part 153-101). The majority of the structural members and couplings of the electrode array (gray parts in figures 1 and 2) were produced from acylonitrile butadiene styrene (ABS) thermoplastic via the Dimension Elite Series three dimensional printer/rapid prototype (Stratasys Inc., Eden Prairie, MN 55344). The structural members were linked together and secured by 10–32 acetyl threaded rods and associated hardware (McMaster-Carr; Robbinsville, NJ 08691-2343).

A similar, larger-scale apparatus was previously proven to measure accurately the dielectric permittivity of different liquids with known values from 4 to 78 (Prodan *et al* 2004, Bot and Prodan 2009, Prodan and Bot 2009).

2.2. Design of the superfusion system

The tissue sample lies between the mobile upper electrode assembly and the stationary lower electrode. The volume between the upper and lower electrodes is the only spatial region within the array that must strictly mimic physiological conditions as closely as possible. The

superfusion system was designed to provide the maximum volume of nutrient-rich and oxygen-saturated electrolytic fluid to the spatial region containing the tissue during characterization. Therefore, two 3/16 inch transverse holes were drilled through the PVC enclosure at 180° of separation at such a height that the holes would be flush with the lower electrode. The directions of the holes were parallel to the plane of the electrode. Two 14 gauge fluorinated ethylene-propylene (FEP) intravenous (IV) catheters with female Leur lock connections were cut to a length of 2.8 mm to correspond to the schedule 40 classification of the PVC pipe. The end of the catheter was inserted through the transverse holes such that the end of the catheter was flush with the inner wall of the PVC enclosure.

As schematically portrayed in figure 2, one catheter serves as the intake port for the nutrient/oxygen-rich solution, which flowed into the characterization chamber through a gravity-fed system whereby a 60 mL syringe served as the electrolyte reservoir and semi-rigid tubing served as the conduit from the reservoir to the intake port. The reservoir was positioned at such a height that the vertical distance between the solution level and the intake port was 120 mm. Solution was continuously added to the reservoir to maintain this 120 mm height. The height of the solution coupled with the inner diameter (1.67 mm) and length (80 mm) of the semi-rigid tubing resulted in an electrolyte flow rate into the chamber of 5.6 mL min^{-1}; this flow rate is considered adequate for tissue survivability (Hajos and Mody 2009). This flow rate was quantified by collecting the volume of Milli-Q water that was allowed to flow through the system in exactly 1 min, taking the mass of that water, and then using the density of the nearly pure water to calculate flow rate. Oxygenation via air stone bubbling directly in the 60 mL reservoir led to a variety of noise errors associated with the formation of oxygen-filled bubbles between the electrodes. Since the relative dielectric permittivity of oxygen is different from that of neural tissue and electrolytic solution, the contribution of the oxygen bubbles are quite apparent, as described later in the paper. For this reason, oxygenation of the experimental electrolytic solution must occur prior to the addition of the solution to the syringe reservoir. To ensure near total oxygen saturation, the top of both the 60 mL reservoir and the larger saline storage container in which oxygenation actually occurs was enclosed. In order to ensure that adequate replacement of the solution within the chamber is achieved, Fast Green dye (Food green #3) is utilized to visualize the constant replacement of solution.

The catheter opposing the intake port serves as the exhaust port for the metabolite/waste-rich solution after contact with the tissue. This waste fluid is drained from the tissue chamber by a variable rate peristaltic pump (VWR 54856-070). The rate of the peristaltic pump was continuously adjusted to match the flow rate of the gravity-fed intake such that the solution level in the PVC enclosure remained constant.

2.3. Preparation of cortical tissue samples

Experiments were performed using a transverse slice preparation of the cerebral cortex of mice; the Animal Care and Facilities Committee at Rutgers University approved this animal protocol. CD-1 mice (Charles River Laboratories) aged from postnatal day 8 (P8) to 16 (P16) with a mean age of 10.75 ± 3.28 days were anesthetized with isoflurane until the absence of a withdrawal reflex from toe pinch. The mice were decapitated and the whole brain dissected in ice cold 'slicing' saline solution containing: 7 mM NaCl, 3 mM KCl, 5 mM HEPES, 30 mM D-glucose, and 276.5 mM sucrose; the pH of this solution was previously adjusted to 7.4. The excised brain was mounted (with cyanoacrylate glue) to an agar block backing with the dorsal side of the brain facing up. Progressive sections of 800 μm thickness were made in the rostral to caudal direction using a vibrating blade microtome (Leica VT 1200). The process of dissection and slicing constitutes a combined hypoxic, ischemic, thermal, and

mechanical trauma to the brain tissue (Richerson and Messer 1995). In order to minimize injury to the tissue as a result of the slicing process, as well as any future injury, the prepared slices were placed in a room temperature 'slicing' saline bath saturated with 100% O_2 until utilized for experimentation.

2.4. Dielectric analysis

The signal analyzer used in this experimental setup has been previously described by Prodan *et al* (2004). Briefly, the tissue is placed in between two electrodes. The output voltage from the signal analyzer is applied to the upper electrode through a resistor of 100 Ω. The bottom electrode is held at a relative ground potential through the negative input of an amplifier. As a result, the current (I) that flows through the sample produces a voltage V_1 that equates to the product of the current (I) and the impedance (Z) of the sample. The voltage V_2 over a reference resistor of 100 Ω is equal to minus the product of the current (I) and resistance (100 Ω). The real and imaginary ratio of these two voltages as a function of frequency was recorded using a Labview program. Therefore, the transfer function (T) is related to the tissue impedance by $T = 100\ \Omega/Z$. If the impedance is not contaminated by the polarization effect, then the complex dielectric function $\varepsilon^* = \varepsilon + \sigma / j\omega$ of the sample can be calculated from

$$\mathrm{Re}\{|Z|\} = \frac{\mathrm{d}\sigma}{\sigma^2 A + \omega^2 \varepsilon_0^2 \varepsilon^2 A}$$

$$\mathrm{Im}\{|Z|\} = \frac{\omega\, \mathrm{d}\varepsilon_0 \varepsilon}{\sigma^2 A + \omega^2 \varepsilon_0^2 \varepsilon^2 A}.$$

3. Results

As aforementioned, superfusion of the tissue is very important and should be done continuously to ensure that the tissue does not deteriorate. However this process can generate air bubbles, which introduce error during the measurements. Figure 3 represents avoidable noise attributed to bubbling 100% oxygen directly into the 60 mL reservoir. Each sharp deviation represents a bubble between the electrodes, which clearly induces error in the measurements. In order to avoid this error, the solution was oxygenated in a 100 mL beaker and incrementally added to the 60 mL syringe at the approximate rate of consumption (6 mL min^{-1}) in order to avoid deoxygenating of the solution. This procedure produced dielectric permittivity spectra with minimal noise, as depicted in figure 6.

If tissue is not superfused and oxygenated, it deteriorates with the passing of time (Hajos and Mody 2009). This deterioration is seen in dielectric measurements as an increase in conductivity due to ions or other charged molecules released into the extracellular solution. Figure 4 (left) shows the conductivity versus frequency for a slice of tissue at various time points without any superfusion. Over the course of 2 h in the same solution the conductivity progressively and significantly increased. However, when the superfusion system is turned on, the conductivity increase is very small as shown in figure 4 (right). Figure 5 represents the conductivity data from figure 4 plotted at a single frequency of 1000 Hz. When no superfusion is present (red line), within 15 min there is a very large increase in the conductivity of the solution and tissue of 0.6 mS m^{-1}. As time increases, conductivity does as well and, after 120 min, there is an increase of 1.5 mS m^{-1} with no significant change after 90 min. In contrast, when the superfusion is turned on (blue line), there is almost no significant increase in the conductivity within the first 20 min. After that, the conductivity started to increase by small amounts compared with the no superfusion case and tends to plateau after 50 min.

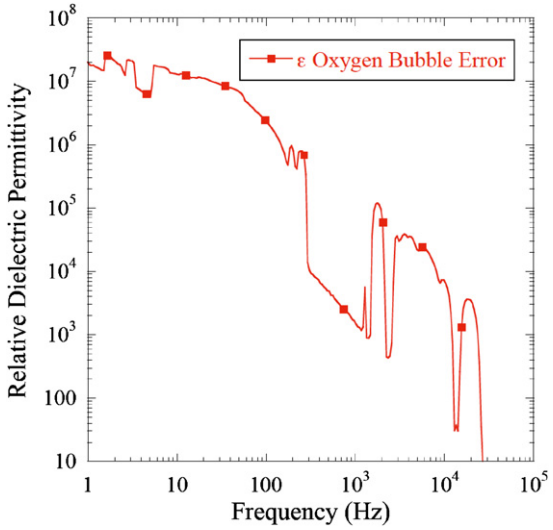

Figure 3. The effect of directly bubbling oxygen into the 60 mL reservoir was apparent in large amounts of noise in the spectra. This error was avoided by oxygenating the solution prior to its placement in the 60 mL syringe.

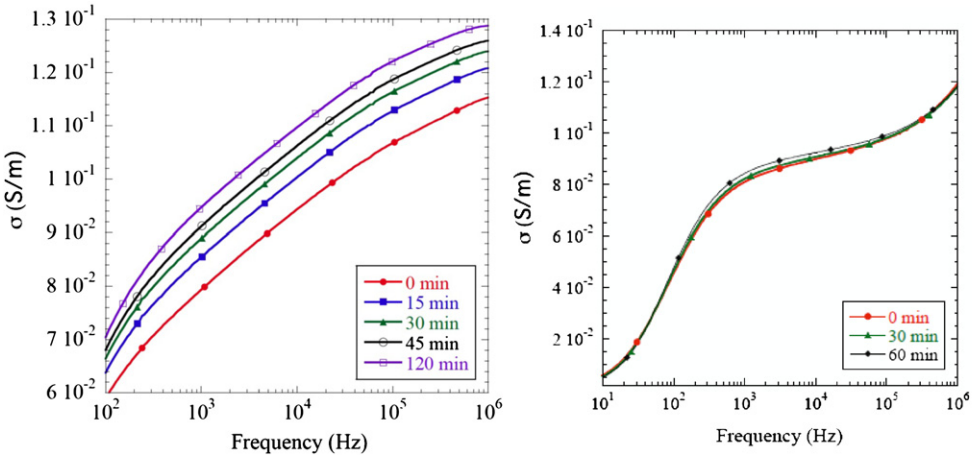

Figure 4. Left: time series of conductivity versus frequency for a slice of tissue without any superfusion. As time increases so does the conductivity of the sample. Right: time series of conductivity verses frequency for a slice of tissue with the superfuison system activated.

Previous researchers (Hajos and Mody 2009) found that the ideal flow rate for proper oxygenation as well as mitigation of forces on the superfused tissue is between 3 and 6 mL min^{-1}. We used the findings of Hajos and Mody and thus maintained the flow rate of the system at 5.6 mL min^{-1}.

Figure 6 represents measurements of the tissue with the superfusion on (blue curve) and immediately after the superfusion was turned off (red curve). The error bars represent standard deviation over four measurements. These measurements were done to ensure the superfusion system has no adverse effects on the dielectric response of the tissue. We concluded that

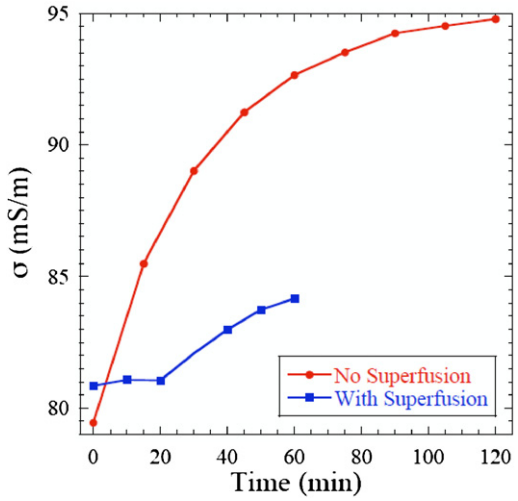

Figure 5. Conductivities from figure 4 plotted at a single frequency of 1000 Hz. One can see the dramatic change in conductivity as the time increase with no superfusion and much less with superfusion.

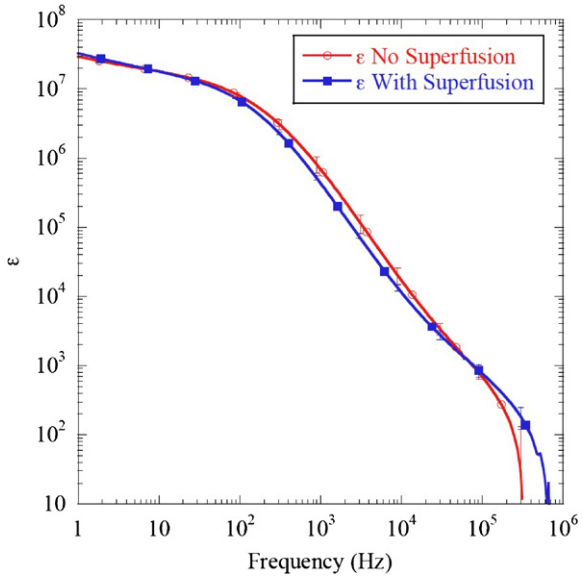

Figure 6. The relative dielectric permittivity spectrums with and without the superfusion system engaged. The overlap of the spectra in several different frequency ranges suggests that the engagement of the superfusion system had no negative effects on the ability to probe the dielectric properties of the tissue. Each curve has 300 or more data points. The symbols are only used to better mark the curves; they do not represent data points.

there are no adverse effects. Moreover, the superfusion system may result in an increase in trial precision and repeatability as represented by the fact that the standard deviations are significantly reduced over the entire frequency spectrum for the superfusion data when compared with the data collected when the superfusion system was inactive.

4. Discussion and conclusions

This paper presents a superfusion method to be used during dielectric spectroscopy measurements to maintain the viability of tissue slices. To keep the tissue healthy during recordings, it has to be continuously oxygenated and provided with key nutrients, such as glucose. Ideally, this process should not interfere with the experiment by creating noise. We investigated several possible methods and found that it was indeed possible to keep the superfusion system active while collecting data. It was concluded that a flow of 3 to 6 mL min^{-1} does not interfere with measurements. If the tissue was not superfused, the conductivity of the solution increased dramatically over time, especially in the first 15 min. Ions and other charged species that are continually being released into the solution could potentially change the dielectric response due to the polarization error and cover the real response of the tissue. When the superfusion system is on, there is no change in the first 15 min, followed by a very small change after that and a plateau after 50 min. The small change could come from the tissue itself.

Another problem with the increase in the ion concentration over time is electrode polarization error. It was determined (Bordi *et al* 2001, Kaatze and Feldman 2006, Prodan and Bot 2009) that even a small increase in the ion concentration increases the polarization error dramatically. The superfusion method utilized here does not remove the polarization error, but it does keep it constant over time since the ions that are released from the brain slice are continuously washed away as the solution is exchanged. By keeping the polarization error constant, the response of the tissue to different pharmaceutical compounds or insults (e.g. hypoxia) may be recorded. Also, the superfusion system could be used as a way to deliver pharmaceutical compounds to the tissue.

Dielectric spectroscopy is a non-invasive technique that may be used to rapidly monitor the electric properties of cells and tissues. While patch clamping remains the 'gold standard' to monitor such properties, it is slow and can be done only one cell at the time. Thus, if the dielectric response of a tissue slice is understood, one can imagine realtime recording from the whole slice. The dielectric properties of cells have been studied and modeled for a long time. It is known that the low frequency dispersion curve is strongly influenced by the membrane potential and by the activity on the ion distribution on the outer part of the membrane (Bot and Prodan 2009). The higher frequency dispersion curve response is influenced by the properties of the membrane and of the cytoplasm, while the highest frequency dispersion curve is dominated by the individual molecules. To obtain useful information, the dielectric response must be recorded from tissue that is maintained in a healthy state even during long experiments. It has been demonstrated that superfusion is critical to maintaining healthy tissue (Haas *et al* 1979).

To conclude, this paper presents a superfusion method that could be used with dielectric spectroscopy measurements of biological tissue. Its use allows the continuous exchange of fresh solution that contains vital nutrients and oxygen, which is known to keep the tissue healthy. This allows measurements of up to several hours on healthy brain tissue.

References

Alger B and Nicoll R 1981 A simple chamber for recording from submerged brain slices *J. Neurosci. Methods* **4** 153–6

Asami K, Yokoi T and Sakoh M 2002 Characterization of solid supported membranes by dielectric spectroscopy: incorporation of channel forming peptides *Membrane* **27** 282–89

Beltran N E, Sanchez-Miranda G, Godinez M, Diaz U and Sacristan E 2006 Gastric impedance spectroscopy in elective cardiovascular surgery patients *Physiol. Meas.* **27** 635

Benjamin H, Bhansali S, Hoath S B, Pickens W L and Smallwood R 2005 A planar micro-sensor for bio-impedance measurements *Sensors Actuators* B **111–112** 430–5

Berardelli A, Rothwell J, Day B and Marsden C 1985 Pathophysiology of blepharospasm and oromandibular dystonia *Brain* **108** 593–608

Bordi T, Cametti C and Gili T 2001 Reduction of the contribution of electrode polarization effects in the radiowave dielectric measurements of highly conductive biological cell suspensions *Bioelectrochemistry* **54** 53–61

Bot C and Prodan C 2009 Probing the membrane potential of living cells by dielectric spectroscopy *Eur. Biophys. J.* **38** 1049–59

Cole K S 1928 Electrical impedance of suspensions of spheres *J. Gen. Physiol.* **12** 29–36

Cole K S 1932 Electrical phase angle of cell membranes *J. Gen. Physiol.* **15** 641–9

Collingridge G L 1995 The brain slice preparation: a tribute to the pioneer Henry McIlwain *J. Neurosci. Methods* **59** 5–9

Dean D A, Ramanathan T, Machado D and Sundararajan R 2008 Electrical impedance spectroscopy study of biological tissues *J. Electrost.* **66** 165–77

Foster K R 2002 Herman P Schwan: a scientist and pioneer in biomedical engineering *Annu. Rev. Biomed. Eng.* **4** 1–27

Foster K R and Schwan H P 1996 Dielectric properties of tissues *Handbook of Biological Effects of Electromagnetic Fields* ed C Polk and E Postow (Boca Raton, FL: CRC Press)

Gabriel C 2007 Dielectric properties of biological materials *Bioengineering and Biophysical Aspects of Electromagnetic Fields* ed F S Barnes and G Ben (Boca Raton, FL: CRC Press)

Gabriel S, Lau R and Gabriel C 1996 The dielectric properties of biological tissues: II. Measurements in the frequency range of 10 Hz to 20 GHz *Phys. Med. Biol.* **41** 2251–69

Gennarelli T A 1986 Mechanisms and pathophysiology of cerebral concussion *J. Head Trauma Rehabil.* **1** 23–9

Gheorghiu E 1993 The resting potential in relation to the equivalent complex permittivity of a spherical cell suspension *Phys. Med. Biol.* **38** 979

Gheorghiu E 1996 Measuring living cells using dielectric spectroscopy *Bioelectrochem. Bioenerg.* **40** 133–9

Gildemesiter M 1912 Über elektrischen Widerstand, Kapazitat und Polarisation der Haut *Arch. Gesamte Physiol.* **176** 84–105

Haas H, Schaerer B and Vosmansky M 1979 A simple perfusion chamber for the study of nervous tissue slices *in vitro J. Neurosci. Methods* **1** 323–5

Hajos N and Mody I 2009 Establishing a physiological environment for visualized *in vitro* brain slice recordings by increasing oxygen supply and modifying aCSF content *J. Neurosci. Methods* **183** 107–13

Hartley D M *et al* 1999 Protofibrillar intermediates of amyloid beta -protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons *J. Neurosci.* **19** 8876–84

Kaatze U and Feldman Y 2006 Broadband dielectric spectroscopy of liquids and biosystems *Meas. Sci. Technol.* **17** R17–35

Kety S 1957 The general metabolism of the brain *in vivo Biochemistry of the Developing Nervous System* ed D Richter (London: Pergamon)

McAdams E T and Jossinet J 1995 Tissue impedance: a historical overview *Physiol. Meas.* **16** A1–13

McRae D A, Esrick M A and Mueller S C 1999 Changes in the noninvasive, *in vivo* electrical impedance of three xenographs during the necrotic cell-response sequence *Int. J. Radiat. Oncol. Biol. Phys.* **43** 849–57

Nightingale A 1958 Medical aspects of non-destructive testing *Progress in Nondestructive Testing* vol 1 ed E G Stanford and J H Fearon (London: Heywood)

Patel P and Markx G H 2008 Dielectric measurement of cell death *Enzyme Microb. Technol.* **43** 463–70

Prodan C and Bot C 2009 Correcting the polarization effect in very low frequency dielectric spectroscopy *J. Phys. D: Appl. Phys.* **42** 175505

Prodan C, Mayo F, Claycomb J, Miller J Jr and Benedik M 2004 Low-frequency, low-field dielectric spectroscopy of living cell suspensions *J. Appl. Phys.* **95** 3754

Raicu V, Saibara T and Irimajiri A 2000 Multifrequency method for dielectric monitoring of cold-preserved organs *Phys. Med. Biol.* **45** 1397–407

Richerson G and Messer C 1995 Effect of composition of experimental solutions on neuronal survival during rat brain slicing *Exp. Neurol.* **131** 133–43

Rigaud B, Morucci J P and Chauveau N 1996 Bioelectrical impedance techniques in medicine: part I. Bioimpedance measurement, second section: impedance spectroscopy *Crit. Rev. Biomed. Eng.* **24** 257–351

Schwan H 1957 Electrical properties of tissue and cell suspensions *Advances in Biological and Medical Physics* ed J L Tobias (New York: Academic)

Seoane F, Lindecrantz K, Olsson T, Kjellmer I, Flisberg A and Bagenholm R 2005 Brain electrical impedance at various frequencies: the effect of hypoxia *Proc. of Engineering and Biology Society, 2004. 26th Annu. Int. Conf. of the IEEE* pp 2322–5

White W, Nadler J and Cotman C 1978 A perfusion chamber for the study of CNS physiology and pharmacology *in vitro Brain Res.* **152** 591–6

Yamamoto C and McIlwain H 1966 Electrical activities in thin sections from the mammalian brain maintained in chemically-defined media *in vitro J. Neurochem.* **13** 1333–43

Ye C, Walsh D M, Selkoe D J and Hartley D M 2004 Amyloid β-protein induced electrophysiological changes are dependent on aggregation state: N-methyl-D-aspartate (NMDA) versus non-NMDA receptor/channel activation *Neurosci. Lett.* **366** 320–5