Bioelectromagnetics 36:603–612 (2015)

Brief Communication

Variation in Dielectric Properties Due to Pathological Changes in Human Liver

Azadeh Peyman,[1]* Bor Kos,[2] Mihajlo Djokić,[3] Blaž Trotovšek,[3] Clara Limbaeck-Stokin,[4] Gregor Serša,[5] and Damijan Miklavčič[2]

[1]*Department of Physical Dosimetry, Centre for Radiation, Chemicals and Environmental Hazards, Public Health England, Didcot, UK*
[2]*Laboratory of Biocybernetics, Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia*
[3]*University Medical Centre Ljubljana, Ljubljana, Slovenia*
[4]*Institute of Pathology, Medical Faculty, University of Ljubljana, Ljubljana, Slovenia*
[5]*Department of Experimental Oncology, Institute of Oncology, Ljubljana, Slovenia*

Dielectric properties of freshly excised human liver tissues (in vitro) with several pathological conditions including cancer were obtained in frequency range 100 MHz–5 GHz. Differences in dielectric behavior of normal and pathological tissues at microwave frequencies are discussed based on histological information for each tissue. Data presented are useful for many medical applications, in particular nanosecond pulsed electroporation techniques. Knowledge of dielectric properties is vital for mathematical calculations of local electric field distribution inside electroporated tissues and can be used to optimize the process of electroporation for treatment planning procedures. Bioelectromagnetics. 36:603–612, 2015. © 2015 Wiley Periodicals, Inc.

Key words: conductivity; permittivity; cancer; pathology; tumor; electroporation

INTRODUCTION

Dielectric spectroscopy has been a useful tool in providing insight into structure and composition of biological tissues. The non-invasive nature of available measurement techniques provides a unique opportunity for detecting physiological and pathological changes with minimum handling of samples. During the last two decades, dielectric data were mainly acquired from normal and healthy tissues and used in numerical simulations of exposure of people to electromagnetic fields (EMF). As technology advances, however, medical applications of EMF to treat cancer tissues have become of higher interest. Therefore, there is need for advanced knowledge of dielectric properties of tissues of different pathological states.

One particular technology becoming more popular is called electroporation, with applications ranging from cancer treatment to gene transfer. It involves exposing biological cells to pulsed electric fields, which results in increased permeability of cellular membranes. Both reversible and irreversible electroporation techniques can be used to either kill cancer cells by applying strong electric pulses or increasing permeability of cellular membrane to enhance cancer drug delivery [Mir et al., 1991; Davalos et al., 2005; Yarmush et al., 2014]. Electroporation-based therapies, such as electrochemotherapy and irreversible electroporation, are already gaining traction in clinical practice, even for treatment of deep-seated tumors [Županič et al., 2012; Miklavčič et al., 2014; Chunlan et al., 2015]. Although most electroporation techniques utilize 100 μs pulses, in recent years there has been a steady drive toward using shorter pulses for treatments (e.g., 100 ns pulse and even as short as

Grant sponsors: Short Term Scientific Mission (STSM); grant number: TD1104-281013-034335; European Network for Development of Electroporation-Based Technologies and Treatments.

Conflict of interest: None.

*Correspondence to: Azadeh Peyman, Department of Physical Dosimetry, Centre for Radiation, Chemicals and Environmental Hazards, Public Health England, Chilton, Didcot OX11 0RQ, UK. E-mail: azadeh.peyman@phe.gov.uk

Received for review 2 April 2015; Accepted 6 October 2015

DOI: 10.1002/bem.21939
Published online 28 October 2015 in Wiley Online Library (wileyonlinelibrary.com).

below 10 ns pulse) [Pakhomov et al., 2007; Nuccitelli et al., 2009, 2010; Arena et al., 2011; Napotnik et al., 2012; Yin et al., 2012; Nuccitelli et al., 2013; Yu-Hsuan et al., 2013; Ibey et al., 2014; Wu et al., 2014].

To optimize the electroporation process for treatment planning procedures, one needs in-depth knowledge of local electric field distribution inside electroporated tissue. A simple yet efficient tool for analysis and explanation of experimental results is mathematical modeling to calculate distribution of an externally applied electric field within tissue of interest. This approach can be used to evaluate electrical phenomena during electroporation in vivo, which is mostly relevant to application of nanosecond pulsed electric fields (nsPEF) for treatments of cells under in vivo conditions [Miklavčič et al., 1998; Miklavčič, 2000; Rems et al., 2013]. Electric field distribution within the tissue is mainly governed by its dielectric characteristics. In the case of in vivo electroporation, there are usually many different tissues/organs with different electrical properties involved. In addition, it is now established that electrical conductivity of cellular membrane (and eventually tissue) increases following application of external electric field [Ivorra et al., 2009; Pakhomov et al., 2010]. Uncertainties in dielectric properties (i.e., electric conductivity of treated tissues and rate of increase in electric conductivity due to electroporation) predefined in numerical models have large effect on electroporation-based therapy and treatment effectiveness [Corovic et al., 2013]. In other words, changes in electrical conductivity due to electroporation need to be taken into account when an electroporation based treatment is planned or investigated. The first step in reducing this uncertainty is to obtain accurate dielectric properties of tissues under treatment before they are electroporated.

In recent years and due to increase in liver tumor cases, many electroporation studies are focused on treatment of liver tumors [Narayanan, 2011; Charpentier, 2012; Kingham et al., 2012]. These studies suggest that there is still a gap in literature for accurate knowledge of dielectric behavior of liver tumors to be used for optimization of treatment plans involving both reversible and irreversible electroporation.

Deformation of normal liver tissue to that of cirrhotic, steatosis, or fibrotic tissues can occur before or during formation of cancer cells. Additionally, primary liver tumors (i.e., hepatocellular carcinoma) mainly occur in cirrhotic livers. This provides a complex combination of pathological state of a single organ tissue. It is, therefore, important to recognize and evaluate differences (if any) in dielectric properties of tissues with different pathological state at microwave frequencies.

Several researchers have reported microwave dielectric properties of normal liver tissues in different species, including those of humans [Stuchly et al., 1982; Joines et al., 1994; Gabriel et al., 1996a,b; Peyman et al., 2001; Stauffer et al., 2003; Lazebnik et al., 2007; O'Rourke et al., 2007]. A few of those studies also reported dielectric properties of human liver carcinoma [Joines et al., 1994; Stauffer et al., 2003; O'Rourke et al., 2007]. However, liver tumor data reported by Joines et al. [1994] only cover up to 900 MHz and are limited to one patient. Stauffer et al. [2003] reported values of two primary and metastatic liver tumors at 915 MHz and observed opposite trends for dielectric properties of tumor tissue and its white mass compared to that of normal tissues. Even though O'Rourke et al. [2007] reported comprehensive dielectric measurements on several normal and malignant human liver tissues covering a wide frequency range (0.05–20 GHz), no distinction has been made between different types of tumors. Authors reported both in vivo and in vitro measured data but found a significant difference between two data sets.

Our work aims to confirm integrity of in vitro dielectric measurements of human liver tumor tissues from several patients to complement data reported by O'Rourke et al. [2007]. Variations in dielectric properties obtained from different patients were examined, and comparisons made between data for different tumor types. Finally, a more in-depth discussion is presented to relate observed trends in dielectric behavior to those of underlying pathological states of tissues.

MATERIALS AND METHODS

Samples

This study was designed to minimize influence on normal clinical procedures and treatment of patients, and it was prepared in accordance with the Declaration of Helsinki. The study proposal was reviewed by the National Medical Ethics Committee of Republic of Slovenia, and approved on December 19, 2013. All patients whose tissue samples were included in the study gave informed consent. Liver slices were taken from six patients during open surgery at the department for abdominal surgery at University Medical Centre of Ljubljana (Ljubljana, Slovenia). Three patients had liver metastasis of adenocarcinoma of stomach or colon. One patient had hemangioma (benign vascular tumor) and one had cirrhotic liver with primary hepatocellular carcinoma. One patient did not have any liver tumors, but

underwent a safety resection due to a gallbladder tumor. Table 1 summarizes patient information and histological details for each sample. Samples were kept in a tight container after removal and no preservative material was used. Time between sample removal and start of dielectric measurements was not longer than 1 h throughout measurement trial. Gross samples were sectioned into roughly 2 cm thick slices, and one or two representative slices containing liver and tumor tissue were measured from each donor. Samples were wiped with cotton tissue to remove blood contamination before dielectric measurements. Figure 1 shows representative images of liver slices containing several segments of tumors. Areas of liver slices, which did not contain a tumor, were considered "normal." However, histological examination revealed that Patient 2 (P2)'s liver sample, there was significant amount of steatosis whereas P3 had a cirrhotic liver. These conditions are not malignant, although they may change dielectric properties of liver tissue.

Dielectric Measurements

Dielectric measurements were carried out in frequency range 50 MHz–5 GHz using an open ended coaxial contact probe technique. Vector network analyzer E5071 and 3.5 mm dielectric measurement probe kit 85070E (Agilent, Santa Rosa, CA) were used. Although measurements were carried out from 50 MHz, due to lack of sensitivity of measurement probe, only data above 100 MHz are reported. The measurement system was calibrated with short circuit, open circuit and standard material (de-ionized water) repeatedly throughout each measurement day to avoid drift. Calibration was checked by measuring standard liquids with well known dielectric characteristics (Formamide [Sigma–Aldrich, St. Louis, MO] and 0.015 M NaCl [B. Braun Melsungen, Melsungen, Germany]) and comparing data with those from literature [Stogryn, 1971; Jordan et al., 1978; Peyman et al., 2007a].

All measurements were carried out at room temperature ($25 \pm 0.5^{\circ}$C), except those for Formamide, which were carried out at 20°C. Temperature of the room, standard liquids, and tissues was monitored using an optical measurement system manufactured by OpSens (Quebec, Canada), consisting of a ProSens signal conditioner and an OTG-F fiber optic temperature sensor. Care was taken to avoid contamination with blood by wiping probe after each measurement and wiping surface blood from samples using cotton tissue.

Uncertainty

Reported typical accuracy of the measurement system is about 5% according to manufacturer. Repeated measurements on Formamide and 0.15 M NaCl solution and comparing results with literature confirmed that measurement accuracy was well within 5% throughout frequency range of interest.

Given availability of a relatively large slice of liver from each patient, several measurements (up to 30) were carried out on each sample at different positions to pool a large number of measured data. Variation of dielectric data on each normal tissue sample was up to 10% but mainly around 5%. Similar variation was observed on measurements on tumor samples, although most variations were around 10%.

TABLE 1. Patient Information and Histological Details of Measured Tumor Samples

Patient number	Sex	Age	Tumor histology	Other observations
P1	Male	61	Metastasis of adenocarcinoma of colon	Liver tissue was significantly steatosis Tumor contained large amount of necrosis
P2	Female	59	Hemangioma	Liver tissue had highest steatosis compared to others in trial Vascular tumor (lots of extracellular space and made from small cells)
P3	Male	65	Hepatocellular carcinoma	Liver tissue was completely changed to cirrhotic—fibrosis condition Tumor type was hepatocellular carcinoma
P4	Male	61	Metastasis of adenocarcinoma of stomach	Liver tissue had very little steatosis Tumor contained more intracellular space
P5	Female	72	No tumor	Liver tissue was free from tumors This was a safety resection due to T2 tumor in gall bladder
P6	Male	62	Metastasis of adenocarcinoma of colon	Liver tissue presented with steato-fibrosis

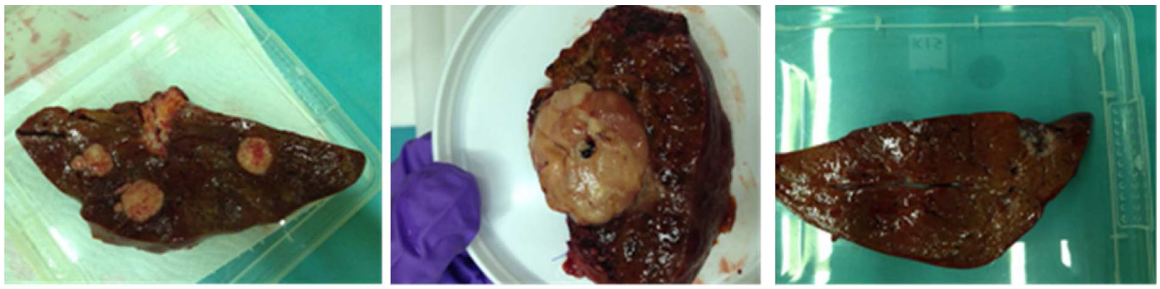

Fig. 1. Examples of liver slices with tumor segments.

According to Gabriel and Peyman [2006], if a dielectric measurement system is free of methodological bias, random fluctuations originating from sampling and natural inhomogeneity dominate the uncertainty budget. In such cases, "mean" value of measured parameter and "standard error of the mean" can be taken as a good measure of the true value and its associated uncertainty.

Curve Fitting

For reader's benefit, we fitted experimental data using a one term Cole–Cole [Cole and Cole, 1941] function in frequency range 0.2–5 GHz:

$$\hat{\varepsilon}(\omega) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + (j\omega\tau)^{1-\alpha}} + \frac{\sigma}{j\omega\varepsilon_0} \qquad (1)$$

where $\hat{\epsilon}$ is complex relative permittivity, ω is angular frequency and Cole–Cole parameters have their usual significance. All parameters were fitted except for ε_∞ value, which was fixed at 4, based on knowledge that corresponding value for water is about 5 and the fact that liver is a high water content tissue. Moreover, previous studies showed that a variation of about 25% in value of ε_∞ has very little effect on other fitted parameters [Peyman and Gabriel, 2010]. Equation (1) is an empirical formulation, not intended for detailed mechanistic investigations but suitable for comparison studies. The reader should also practice caution in extrapolating fitted parameters to frequencies outside the range reported here.

Analysis was carried out using a complex curve-fitting program (FORTRAN) using iteration and least-squares minimization of root mean square error or sum of squared residuals (residual being difference between observed values and values provided by model). Differences across frequency range (200 MHz–5 GHz) between measured and fitted values were mainly around 2–3%. Data for each sample and each tissue type were fitted separately. Examples of measured versus fitted plots are presented in Figure 2 whereas fitted parameters are summarized in Table 2.

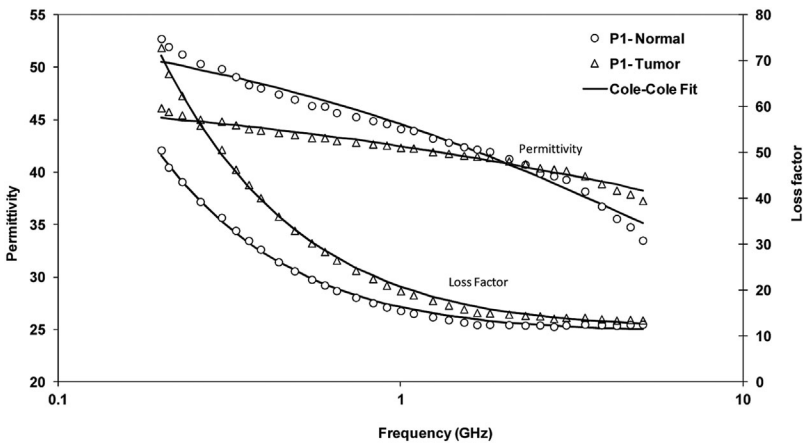

Fig. 2. Measured vs fitted permittivity and loss factor for normal and tumor tissues taken from Patient 1.

TABLE 2. Average Parameters From Cole–Cole Fit to Multiple Measurements on Different Tissue Samples

	Histology	ε_s	τ (*ps*)	σ (s/m)	α	Root mean square error
Patient 1						
Normal, $n=28$	Some steatosis	56.72 ± 2.12	15.60 ± 4.32	0.50 ± 0.01	0.54 ± 0.04	1.01
Tumor 28, $n=28$	Metastasis of adenocarcinoma of colon	64.49 ± 2.58	10.93 ± 3.20	0.74 ± 0.01	0.57 ± 0.05	1.14
Tumor (necrosis), $n=3$	Tumour contained large amount of necrosis	51.00 ± 2.38	14.40 ± 5.37	0.55 ± 0.01	0.57 ± 0.05	0.98
Patient 2						
Normal, $n=16$	Most steatosis	43.71 ± 0.48	18.65 ± 3.17	0.41 ± 0.01	0.45 ± 0.01	0.82
Tumor, $n=7$	Hemangioma	63.64 ± 3.13	8.93 ± 3.15	0.80 ± 0.02	0.50 ± 0.08	2.00
Patient 3						
Normal, $n=9$	Cirrhosis	60.98 ± 1.77	12.77 ± 2.57	0.55 ± 0.01	0.53 ± 0.03	0.86
Tumor, $n=9$	Hepatocellular carcinoma	59.89 ± 2.35	11.77 ± 3.09	0.65 ± 0.02	0.50 ± 0.06	1.41
Patient 4						
Normal, $n=20$	Least steatosis	57.39 ± 2.03	10.54 ± 2.58	0.54 ± 0.01	0.52 ± 0.05	1.18
Tumor, $n=10$	Metastasis of adenocarcinoma of stomach	67.74 ± 1.95	6.53 ± 1.55	0.78 ± 0.01	0.52 ± 0.05	1.28
Patient 5						
Normal, $n=20$	None	59.42 ± 1.99	9.63 ± 2.26	0.55 ± 0.01	0.52 ± 0.05	1.21
Patient 6						
Normal, $n=30$	None	58.64 ± 1.79	8.54 ± 1.88	0.55 ± 0.01	0.52 ± 0.05	1.11
Tumor, $n=17$	Metastasis of adenocarcinoma of colon	64.59 ± 0.77	5.33 ± 1.13	0.95 ± 0.01	0.39 ± 0.04	0.94

n, number of measurements on each tissue sample.

RESULTS AND DISCUSSION

Normal Liver Tissues

Figures 3a and b show measured permittivity and conductivity of normal liver tissues from all six patients. Available literature data were also added for comparison. Results show that measured dielectric properties of normal liver tissue from different patients correlate well except for that of P2. Several measurements on P2's normal liver sample consistently returned much lower values of permittivity and conductivity compared to other samples in this trial. Histological data reveals that P2 had the most pronounced steatosis (a.k.a., fatty liver disease—lipid accumulation in liver cells); it is, therefore, logical to conclude that the fatty state of P2's liver may have caused significantly lower dielectric values compared to others. In fact, a trend can be established between the extent of steatosis state of each liver tissue and measured dielectric properties. P1's tissue also had some steatosis (though not as much as P2), hence, lower dielectric values than others. Dielectric data presented for P2 can be used as an indicator for advanced steatosis case and has potential to be used as a detecting tool.

Dielectric data collected in this trial for normal liver tissue agree quite well with those reported by Joines et al. [1994], Gabriel et al. [1996b], Stauffer et al. [2003] (in vitro), and Peyman et al. [2005] (in vivo). The in vitro data of O'Rourke et al. [2007] were generally on higher side compared to all other reported values. Moreover, their in vivo data were even higher than any other data reported in the literature, even those reported under similar conditions by Peyman et al. [2005]. O'Rourke et al. [2007] were not able to explain the reason for significantly higher values of dielectric properties reported for in vivo. They ruled out experimental artefacts and indicated a possible biophysical mechanism responsible for these differences. On the contrary, Peyman et al. [2005] and [2007b] ascertained that if care was taken to avoid contamination with body fluid, in particular blood, during in vivo measurements, and if tissues did not lose their moisture content during in vitro measurements, results of two data sets would not be significantly different at microwave frequencies. Systematic dielectric measurements (both in vivo and in vitro) on several porcine tissues under strict temperature control revealed that sampling in vivo is difficult and problems such as partial contact and body fluid oozing at measurement site could affect measurements in opposite directions [Peyman et al., 2005, 2007b].

Measured dielectric properties of P3's cirrhotic liver were on the higher side compared to other normal samples. O'Rourke et al. [2007] also observed

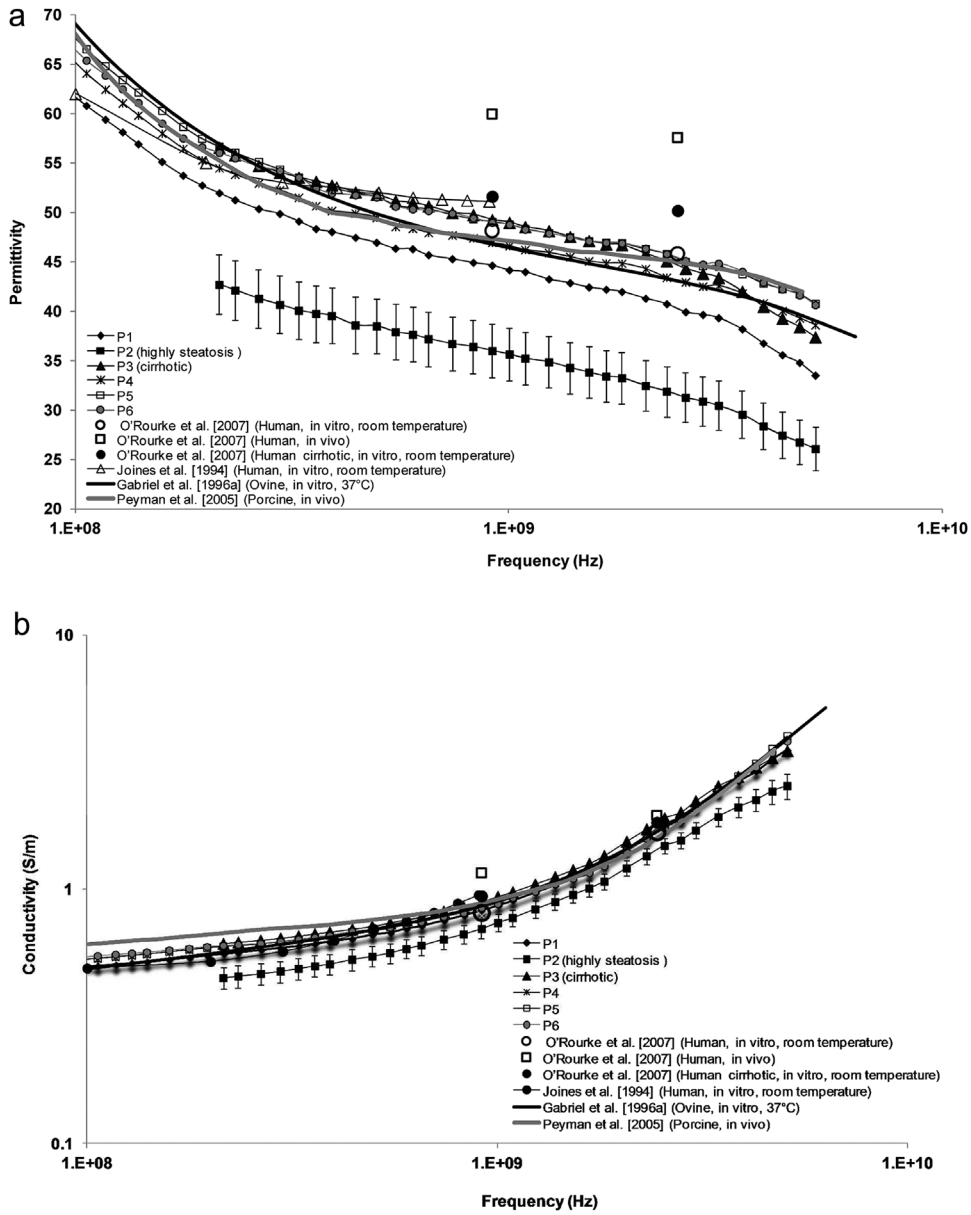

Fig. 3. Measured (**a**) permittivity and (**b**) conductivity of normal liver tumors (P1-P6) in comparison with literature data. Error bars for P2 plot represent standard deviation of several measurements. Error bars for other plots are removed for clarity.

significantly higher values for cirrhotic liver but did not draw a general conclusion as sample size for cirrhotic liver in their experiment was small. This correlation may be indicative of a systematic trend in dielectric properties of cirrhotic liver compared to that of normal tissue.

The above observation suggests that normal tissues from all patients can be pooled together (except those of P2) to use for comparison with tumor data. Finally, one can conclude from data presented in Figures 3a and b that variations between dielectric properties of normal liver tissue from different species and at a range of temperatures (room temperature to 37°C) are not greater than variation from one sample to another due to natural inhomogeneity of tissues.

Tumor Tissues

For each patient, measured dielectric properties (both permittivity and conductivity) of tumor seg-

ments were significantly higher than those of normal tissue (except for P3 with cirrhotic liver). This trend is expected and reported in literature previously [Joines et al., 1994; O'Rourke et al., 2007]. Higher dielectric properties of tumor tissues could be attributed to the fact that cancer cells generally contain higher sodium and water content than normal cells [Cone, 1970, 1975; Cope, 1978]. This is because cancer cells have altered membrane composition and membrane permeability, which results in movement of potassium, magnesium, and calcium out of cell and accumulation of sodium and water into cell [Seeger and Wolz, 1990].

Figures 4a and b show measured permittivity and conductivity of different tumors compared to those obtained and pooled from several normal livers.

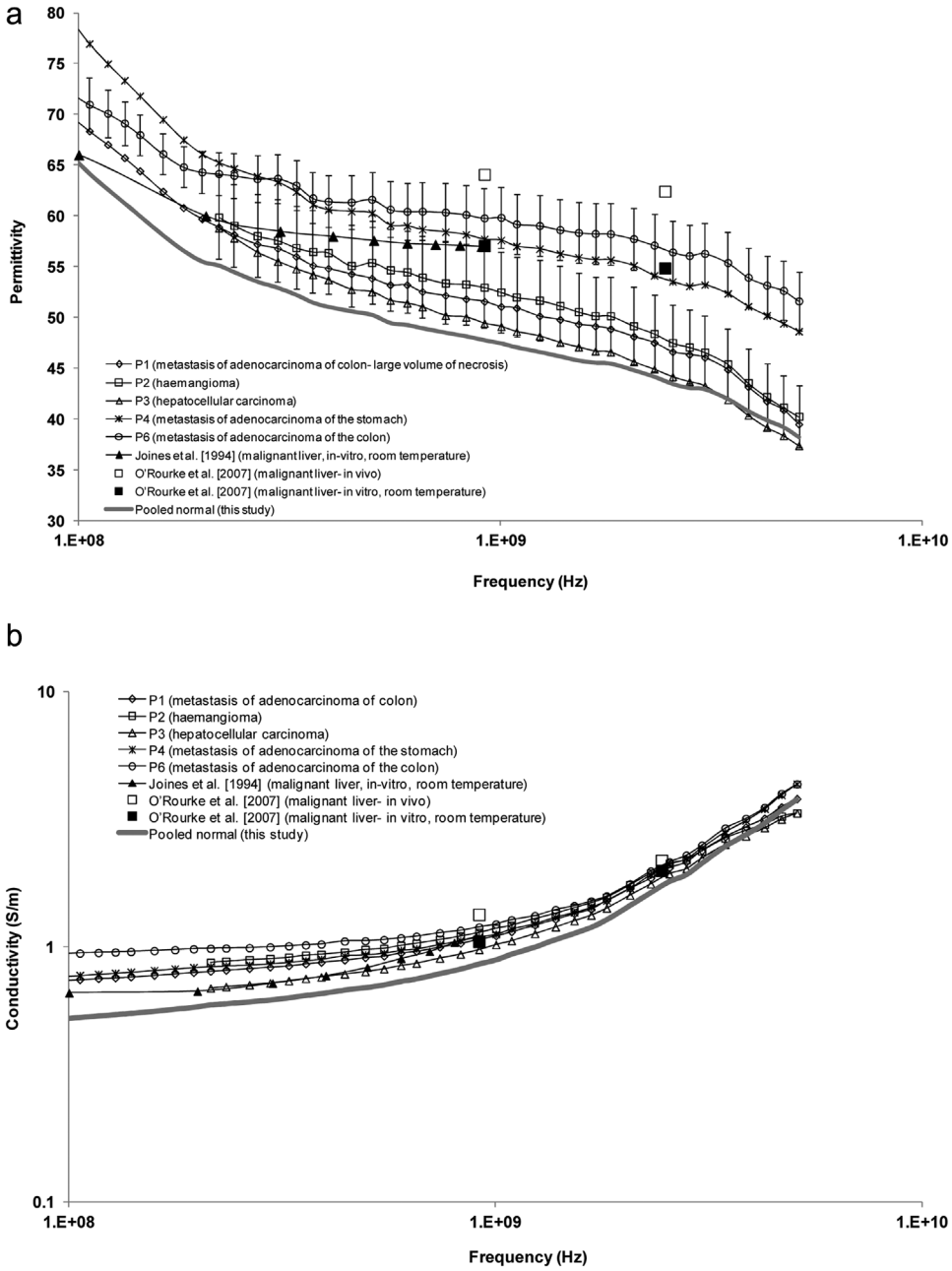

Fig. 4. Measured (**a**) permittivity and (**b**) conductivity of liver tumors (P1-P6) in comparison to literature data. Error bars for P2 and P6 plots represent standard deviation of several measurements. Error bars for other plots are removed for clarity.

For comparison, data reported for malignant liver by Joines et al. [1994] and O'Rourke et al. [2007] were also added.

As evident from Figure 4a, permittivity values of tumors measured in this study can be grouped into two. Liver tumors from P4 and P6 resulted from metastasis of adenocarcinoma of colon and stomach and both had relatively higher values of permittivity compared to other tumors. P4's tumor was highly cellular with some fibrous stroma. P1 also had a tumor formed in the liver as a result of adenocarcinoma of colon; however, the tumor contained a large volume of necrotic (dead) tissue resulting in much lower permittivity values.

Permittivity of hemangioma tumor (P2) seemed to be higher than that of hepatocellular carcinoma (P3). This trend may be explained by the fact that hemangioma is a vascular tumor made from small cells with lots of extracellular spaces filled with electrolyte fluid.

The literature data from Joines et al. [1994] and O'Rourke et al. [2007] (in vitro) for malignant tissue (no type was specified) lie somehow between the two grouped data sets. Similar to that of normal liver, data collected by O'Rourke et al. [2007] for malignant liver under in vivo conditions were much higher than all recorded data shown in Figure 4a, indicating possible contamination with blood and other body fluids.

A similar trend can be observed for measured conductivity values as shown in Figure 4b with the exception of hemangioma tumor (P2) that had relatively high conductivity values close to those of P6, which may be explained by the fact that it grows from blood vessels and larger water content of hemangioma.

Table 2 contains Cole–Cole parameters for all measured samples, where differences in static permittivity and conductivity are clearly shown.

As expected, static permittivity values for normal tissues extracted from different patients are comparable and are close to those reported by O'Rourke et al. [2007]. P2's liver was severely affected by steatosis and P3 had a cirrhotic liver. These conditions correlate with different values of static permittivity compared to those of normal tissues. A cirrhotic liver has much higher static permittivity than normal tissue, whereas a liver with steatosis has significantly lower static permittivity due to high level of fatty cells content. In all cases, static permittivity of tumor tissue is much higher than that of normal tissue. When the tumor becomes necrotic (as in P1), static permittivity drops even below normal tissue value.

A similar trend can be observed for static conductivity values, whereas tumor tissues have much higher conductivity than those of normal tissues. Liver with diffuse steatosis (P2) has lowest conductivity as expected due to higher fat content as described above. Conductivity of cirrhotic liver is somewhere between normal and tumor tissues, closer to tumor values. An observed trend in this study, however, is somehow different in comparison with those reported by O'Rourke et al. [2007] where the conductivity of tumor and normal tissues are relatively close and significantly lower than that of cirrhotic liver. O'Rourke et al. [2007] did not provide any explanation for this observation.

CONCLUSION

Microwave dielectric measurements were performed on several normal and malignant human liver tissues at room temperature and results revealed similar values to those previously reported. Furthermore, variation between dielectric properties of normal human liver tissue and those of other species at a range of temperatures are not greater than variation due to natural inhomogeneity of each tissue.

In terms of dielectric properties, categorizing liver tissue to normal and malignant may not be as clear cut as other pathological conditions such as cirrhosis and steatosis, which can occur in livers that are not necessarily malignant. This study confirms that cirrhotic liver has higher permittivity and conductivity values compared to normal liver. Replacement of liver tissue by fibrosis and edema due to higher resistance to blood flow from portal vein through sinusoids toward vena cava can be the reason for increased dielectric properties of cirrhotic liver.

On the other hand, steatosis (fatty liver disease) results in significant decline in both permittivity and conductivity of liver tissue. This study presents a trend between extent of steatosis in liver tissue and measured dielectric properties, which may be used as a detecting tool for advanced steatosis.

As expected, liver tumors had higher dielectric properties compared to normal baseline. Higher sodium and water content of cancer cells, altered membrane composition and membrane permeability, and accumulation of sodium and water into cancer cells can attribute to higher permittivity and conductivity of tumor samples.

Furthermore, dielectric characterization of tumors varies by tumor type. For instance, hemangioma tumor has higher permittivity and higher conductivity compared to hepatocellular carcinoma (HCC) due to its vascular origin and increased

extracellular space, whereas metastatic liver tumors such as adenocarcinoma of colon and stomach show even higher dielectric properties compared to HCC.

Detailed dielectric data reported for normal and malignant liver tissues can be used in mathematical modeling to calculate distribution of externally applied electric field within a tissue. This approach can be used to evaluate and optimize electroporation applications using nsPEF for treatments of cells under in vivo condition.

ACKNOWLEDGMENTS

We would like to thank professor Dean Ravnik and professor Marjana Horvat from Institute for Anatomy of medical faculty of the University of Ljubljana for generously allowing us to use their lab space to perform our measurements. We gratefully acknowledge Department of Biochemistry and Chemistry of Foodstufs, led by professor Nataša Poklar Ulrih for lending us the network analyzer and dielectric probe used in measurements. Thanks are also due to Dr. Camelia Gabriel from MCL-P for useful discussions on analysis of dielectric data.

REFERENCES

Arena CB, Sano MB, Rossmeisl JH, Caldwell JL, Garcia PA, Rylander MN, Davalos RV. 2011. High-frequency irreversible electroporation (H-FIRE) for non-thermal ablation without muscle contraction. Biomed Eng Online 10:102.

Charpentier KP. 2012. Irreversible electroporation for the ablation of liver tumors are we there yet? Arch Surg 147:1053–1061.

Chunlan J, Davalos R, Bischof J. 2015. A review of basic to clinical studies of irreversible electroporation therapy. IEEE Trans Biomed Eng 62:4–20.

Cole KS, Cole RH. 1941. Dispersion and absorption in dielectrics I. alternating current characteristics. J Chem Phys 9:341–352.

Cone CD. 1970. Variation of the transmembrane potential level as a basic mechanism of mitosis control. Oncology 24:438–470.

Cone CD. 1975. The role of surface electrical transmembrane potential in normal and malignant mitogenesis. Ann NY Acad Sci 238:420–435.

Cope FW. 1978. A medical application of the Ling Association-Induction Hypothesis: The high potassium, low sodium diet of the Gerson cancer therapy. Physiol Chem Phys 10:465–468.

Corovic S, Lackovic I, Sustaric P, Sustar T, Rodic T, Miklavčič D. 2013. Modeling of electric field distribution in tissues during electroporation. Biomed Eng Online 12:16.

Davalos R, Mir L, Rubinsky B. 2005. Tissue ablation with irreversible electroporation. Ann Biomed Eng 33:223–231.

Gabriel C, Peyman A. 2006. Dielectric measurement: Error analysis and assessment of uncertainty. Phys Med Biol 51:6033–6046.

Gabriel S, Lau R, Gabriel C. 1996a. The dielectric properties of biological tissues: III. Parametric models for the dielectric spectrum of tissues. Phys Med Biol 41:2271–2293.

Gabriel S, Lau R, Gabriel C. 1996b. The dielectric properties of biological tissues: II. Measurements in the frequency range 10Hz to 20GHz. Phys Med Biol 41:2251–2269.

Ibey BL, Ullery JC, Pakhomova ON, Roth CC, Semenov I, Beier HT, Tarango M, Xiao S, Schoenbach KH, Pakhomov AG. 2014. Bipolar nanosecond electric pulses are less efficient at electropermeabilization and killing cells than monopolar pulses. Biochem Biophys Res Commun 443:568–573.

Ivorra A, Al-Sakere B, Rubinsky B, Mir LM. 2009. In vivo electrical conductivity measurements during and after tumor electroporation: conductivity changes reflect the treatment outcome. Phys Med Biol 54:5949–5963.

Joines WT, Zhang Y, Li C, Jirtle RL. 1994. The measured electrical properties of normal and malignant human tissues from 50 to 900MHz. Med Phys 21:547–550.

Jordan BP, Sheppard RJ, Szwarnowski S. 1978. The dielectric properties of formamide, ethanediol and methanol. J Phys Appl Phys 11:695–701.

Kingham TP, Karkar AM, D'Angelica MI, Allen PJ, DeMatteo RP, Getrajdman GL, Sofocleous CT, Solomon SB, Jarnagin WR, Fong Y. 2012. Ablation of perivascular hepatic malignant tumors with irreversible electroporation. J Am Coll Surg 215:379–387.

Lazebnik M, Popovic D, McCartney L, Watkins CB, Lindstrom MJ, Harter J, Sewall S, Ogilvie T, Magliocco A, Breslin TM, Temple W, Mew D, Booske JH, Okoniewski M, Hagness SC. 2007. A large-scale study of the ultrawideband microwave dielectric properties of normal, benign and malignant breast tissues obtained from cancer surgeries. Phys Med Biol 52:6093–6115.

Miklavčič D. 2000. Numerical modeling for in vivo electroporation. Methods Mol Med 37:63–81.

Miklavčič D, Beravs K, Šemrov D, Čemažar M, Demšar F, Serša G. 1998. The importance of electric field distribution for effective in vivo electroporation of tissues. Biophys J 74:2152–2158.

Miklavčič D, Mali B, Kos B, Heller R, Serša G. 2014. Electrochemotherapy: From the drawing board into medical practice. Biomed Eng Online 13:29.

Mir LM, Orlowski S, Belehradek J, Paoletti C. 1991. Electrochemotherapy potentiation of antitumor effect of bleomycin by local electric pulses. Eur J Cancer 27:68–72.

Napotnik TB, Wu YH, Gundersen MA, Miklavčič D, Vernier PT. 2012. Nanosecond electric pulses cause mitochondrial membrane permeabilization in Jurkat cells. Bioelectromagnetics 33:257–264.

Narayanan G. 2011. Irreversible electroporation for treatment of liver cancer. Gastroenterol Hepatol (NY) 7:313–316.

Nuccitelli R, Chen X, Pakhomov AG, Baldwin WH, Sheikh S, Pomicter JL, Ren W, Osgood C, Swanson RJ, Kolb JF, Beebe SJ, Schoenbach KH. 2009. A new pulsed electric field therapy for melanoma disrupts the tumor's blood supply and causes complete remission without recurrence. Int J Cancer 125:438–445.

Nuccitelli R, Tran K, Sheikh S, Athos B, Kreis M, Nuccitelli. P. 2010. Optimized nanosecond pulsed electric field therapy can cause murine malignant melanomas to self-destruct with a single treatment. Int J Cancer 127:1727–1736.

Nuccitelli R, Huynh J, Lui K, Wood R, Kreis M, Athos B, Nuccitelli P. 2013. Nanoelectroablation of human pancreatic carcinoma in a murine xenograft model without recurrence. Int J Cancer 132:1933–1939.

O'Rourke AP, Lazebnik M, Bertram JM, Converse MC, Hagness SC, Webster JG, Mahvi DM. 2007. Dielectric properties of

human normal, malignant and cirrhotic liver tissue: in vivo and ex vivo measurements from 0.5 to 20GHz using a precision open-ended coaxial probe. Phys Med Biol 52:4707–4719.

Pakhomov AG, Kolb JF, White JA, Joshi RP, Xiao S, Schoenbach KH. 2007. Long-lasting plasma membrane permeabilization in mammalian cells by nanosecond pulsed electric field (nsPEF). Bioelectromagnetics 28:655–663.

Pakhomov AG, Miklavčič D, Markov MS. 2010. Advanced electroporation techniques in biology and medicine. Boca Raton, FL: CRC Press, Taylor and Francis Group.

Peyman A, Gabriel C. 2010. Cole–Cole parameters for the dielectric properties of porcine tissues as a function of age at microwave frequencies. Phys Med Biol 55: N413–N419.

Peyman A, Rezazadeh AA, Gabriel C. 2001. Changes in the dielectric properties of rat tissue as a function of age at microwave frequencies. Phys Med Biol 46:1617–1629.

Peyman A, Holden S, Gabriel C. 2005. Dielectric properties of tissues at microwave frequencies. Mobile Telecommunications and Health Research Programme Final Report, http://www.mthr.org.uk/research_projects/documents/Rum3FinalReport.pdf Chilton, Didcot, Oxfordshire, UK [Last accessed 30 Sept 2015].

Peyman A, Gabriel C, Grant EH. 2007a. Complex permittivity of sodium chloride solutions at microwave frequencies. Bioelectromagnetics 28:264–274.

Peyman A, Holden S, Watts S, Perrott R, Gabriel C. 2007b. Dielectric properties of porcine cerebrospinal tissues at microwave frequencies: in vivo, in vitro and systematic variation with age. Phys Med Biol 52:2229–2245.

Rems L, Ušaj M, Kandušer M, Reberšek M, Miklavčič D, Pucihar G. 2013. Cell electrofusion using nanosecond electric pulses. Sci Rep 3:3382.

Seeger PG, Wolz S. 1990. Successful biological control of cancer by combat against the causes. Melsbach, Germany: Neuwieder Verlagsgesellschaft.

Stauffer PR, Rossetto F, Prakash M, Neuman DG, Lee T. 2003. Phantom and animal tissues for modelling the electrical properties of human liver. Int J Hyperth Off J Eur Soc Hyperthermic Oncol North Am Hyperth Group 19:89–101.

Stogryn A. 1971. Equations for calculating the dielectric constant of saline water (correspondence). IEEE Trans Microw Theory Tech 19:733–736.

Stuchly MA, Kraszewski A, Stuchly SS, Smith AM. 1982. Dielectric properties of animal tissues in vivo at radio and microwave frequencies: Comparison between species. Phys Med Biol 27:927–936.

Wu S, Wang Y, Guo J, Chen Q, Zhang J, Fang J. 2014. Nanosecond pulsed electric fields as a novel drug free therapy for breast cancer: an in vivo study. Cancer Lett 343:268–274.

Yarmush ML, Golberg A, Serša G, Kotnik T, Miklavčič D. 2014. Electroporation-based technologies for medicine: Principles, applications, and challenges. Annu Rev Biomed Eng 16:295–320.

Yin D, Yang WG, Weissberg J, Goff CB, Chen W, Kuwayama Y, Leiter A, Xing H, Meixel A, Gaut D, Kirkbir F, Sawcer D, Vernier PT, Said JW, Gundersen MA, Koeffler HP. 2012. Cutaneous papilloma and squamous cell carcinoma therapy utilizing nanosecond pulsed electric fields (nsPEF). PLoS ONE 7:e43891.

Yu-Hsuan W, Arnaud-Cormos D, Casciola M, Sanders JM, Leveque P, Vernier PT. 2013. Moveable wire electrode microchamber for nanosecond pulsed electric-field delivery. IEEE Trans Biomed Eng 60:489–496.

Županič A, Marčan M, Serša G, Miklavčič D. 2012. Patient-specific treatment planning of electrochemotherapy: Procedure design and possible pitfalls. Bioelectrochemistry 87:265–273.