Ex vivo electrical impedance measurements on excised hepatic tissue from human patients with metastatic colorectal cancer

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Abstract
Point-wise ex vivo electrical impedance spectroscopy measurements were conducted on excised hepatic tissue from human patients with metastatic colorectal cancer using a linear four-electrode impedance probe. This study of 132 measurements from 10 colorectal cancer patients, the largest to date, reports that the equivalent electrical conductivity for tumor tissue is significantly higher than normal tissue ($p < 0.01$), ranging from 2–5 times greater over the measured frequency range of 100 Hz–1 MHz. Difference in tissue electrical permittivity is also found to be statistically significant across most frequencies. Furthermore, the complex impedance is also reported for both normal and tumor tissue. Consistent with trends for tissue electrical conductivity, normal tissue has a significantly higher impedance than tumor tissue ($p < 0.01$), as well as a higher net capacitive phase shift (33° for normal liver tissue in contrast to 10° for tumor tissue).

Keywords: impedance, liver, tumor, colorectal cancer, metastasis

(Some figures may appear in colour only in the online journal)
1. Introduction

The established, state-of-the-art practice (van der Vorst et al 2013) for the treatment of liver lesions, including solid malignancies, is resection, dating back to the first reported case in 1888 (Ballantyne and Quin 1993). It is well known that 60–70% of the patients with primary colorectal cancer (CRC) will ultimately develop metastatic disease, with 20–35% of patients having isolated liver metastases (Tomlinson et al 2007). Among the typical treatment options are surgical resection and thermal ablation therapy performed at radio frequencies (RF) to cause localized heating for tissue destruction using electrodes placed directly within the tumor (Rossi et al 1996, Tanaka et al 2006, Haemmerich et al 2009). Optimization of RF thermal ablation depends upon the availability of detailed and accurate electrical properties of tissues, and these properties can also be of use in detection of the tumor for diagnostics purposes. In fact, knowledge of tissue electrical properties has been used to distinguish between normal tissue and tumor tissue for breast cancer and cervical carcinomas (Kerner et al 2002, Miranda et al 2012, Karpov et al 2013).

An extensive search of literature yielded only two previous studies that report electrical properties of cancerous human hepatic tissue (Haemmerich et al 2009, Laufer et al 2010) in the 1 Hz–1 MHz probing frequency range, with other previous studies (Geddes and Baker 1967, Gabriel et al 1996, 2009) presenting a compilation of normal tissue data from human and animal sources or a statistical meta-analysis of previously published values (Faes et al 1999). The 1 Hz–1 MHz range of probing frequencies is important for a variety of reasons, including treatment methodologies, such as RF ablation (400–500 kHz) used to treat tumors, as well as the fact that ionic species transport, and polarization are known to occur in biological tissues between 1 Hz–1 MHz (Foster and Schwan 1989, Gabriel et al 1996, Curley et al 1997).

The first of the two previous studies to report electrical properties for cancerous liver tissue (Haemmerich et al 2009) comprised 6 patients with liver metastases from colorectal cancer for a total of 8 tumor tissue and 6 normal tissue measurements. The data was reported as an equivalent electrical conductivity of the tissue using a probe calibration against a 0.9% saline solution at room temperature with frequency range for the measurements from 10 Hz to 1 MHz. The most recent study reporting human liver data from cancer patients, conducted in the 1–400 kHz range (Laufer et al 2010), also reports data from 6 patients. Of these 6 patients, four patients had liver metastases from CRC and two patients had primary liver cancer for a total of 26 measurement points on normal liver tissue (non-tumor), 32 measurements on cancerous liver, and 7 measurements on cirrhotic liver tissue. The data were presented as equivalent electrical conductivities and permittivities of the tissues using probe calibration against NaCl solutions of known concentration ranging from 0.001 to 0.15 M. (Laufer et al 2010).

There has been extensive use of impedance measurements and development of related technology for use in diagnostics and treatment of tissue-based pathologies (Fricke and Morse 1926, Pethig and Kell 1987, Haemmerich et al 2003, Zou and Guo 2003, Bayford 2006, Halter et al 2007, Dean et al 2008, Wang et al 2013, Karki et al 2014). Therefore, it is essential to develop a large database of human tissue data, for both normal and abnormal tissue states to meet the anticipated needs of emerging treatment methods (Laufer et al 2010) and to advance the understanding of metastatic disease in liver tissue. The purpose of this paper is to report impedance measurements and derived equivalent electrical conductivity and permittivity values from liver metastases and surrounding normal liver tissue from patients with metastatic CRC. The data presented here is from excised remnant liver tissue from 10 CRC patients undergoing surgical resection for liver metastases, for a total of 91 measurements on normal liver tissue and 41 measurements on metastatic tumor tissue. The data here represents the
largest number of patients and impedance measurements on cancerous human hepatic tissue reported to date.

2. Methods and materials

In this work, data is reported from 10 patients (7 male, 3 female) between the ages of 34 and 65, for a total of 91 measurement locations in normal (non-tumor) tissue (classified as no visually identifiable tumor present) and 41 measurement locations in tumor tissue. All data were collected as part of a study approved by the Cancer Institutional Review Board (IRB) at The Ohio State University and The Ohio State University Comprehensive Cancer Center Clinical Scientific Review Committee. Post resection, tissue was immediately registered with the Department of Pathology, where relevant tissue was sectioned for clinical pathological assessment. The measurements reported here were conducted on remnant tissue. The sample sizes obtained for the measurements reported here ranged from the largest sample being 21 cm long, 9 cm wide, and 2 cm thick, to the smallest sample being 3 cm long, 2 cm wide, and 1 cm thick. Impedance measurements were conducted within 90 min of the tissue being exposed to air in order to minimize tissue desiccation. Additionally, a time study on one human liver sample with 61 measurements at a single location over a 90 min period showed an average measured
tissue impedance variance of ~10% across all 61 measurements. Overall, the measurement protocols follow methodologies consistent with those reported previously (Haemmerich et al 2009, Laufer et al 2010).

The electrical or electrochemical impedance spectroscopy (EIS) measurements were performed using a 4-electrode probe (Schwan and Kay 1957, Bard and Faulkner 1980, Tsai et al 2000, 2002, Kerper et al 2002, Haemmerich et al 2003, Halter et al 2007, Haemmerich et al 2009, Laufer et al 2010, Vitarelli et al 2011). Electrodes were made using 99.9% pure Platinum (Pt) (Alfa Aesar, MA, USA), which is known to have a reliable performance in ac measurements (Schwan 1968). The electrodes were arranged in a linear configuration with the experimental set-up shown in figure 1. Before measurements were made on each excised tissue sample, the impedance probe was cleaned using the RCA-1 cleaning procedure (Prakash et al 2007, Prakash and Karacor 2011, Vitarelli et al 2011), where the probe is soaked in a 100:10:1 solution of H2O:H2O2:NH4OH at 73 °C for 30 min followed by a de-ionized (DI) water rinse. The probe is then dried in a stream of filtered compressed air and stored in a sealed container until further use. After completing measurements on each tissue sample, the impedance probe was soaked in a 10% bleach solution for 30 min, rinsed with DI water, dried with filtered air, and then stored in a sealed container until further use.

The EIS measurements were completed using a potentiostat (Interface 1000 from Gamry Instruments, PA, USA) in the galvanostatic mode with 30 µA rms injection current over a frequency range of 100 Hz–1 MHz with 10 points per decade for a total of 41 frequencies. The selection of this current level was based on a related study investigating the change between subsequent EIS measurements with varying levels of input current in porcine muscle tissue. This related study showed that 30 µA rms can be used to produce an adequate signal without permanently altering tissue electrical properties. The frequency range was chosen to include several frequencies in the typical RF ablation range, as well as frequencies critical to fundamental transport phenomena in tissue within the commonly defined ‘β’ dispersion (Foster and Schwan 1989).

As the EIS probe comprises Pt electrodes and it is known that low frequencies induce electrode polarization, only frequencies above 100 Hz are considered in this work (Schwan 1963, Foster et al 1976, Laufer et al 2010, Barthel et al 2012). Each 4-electrode probe was made by passing individual Pt wires (referred to as electrodes in subsequent discussions) through an acrylic block, extending 6 mm beyond the bottom surface and held in place by commercial epoxy (JB Kwik Weld Epoxy). A thin wall polyolefin shrink tubing (NTE Electronics, Inc., NJ, USA) was used to insulate 2 mm length of the electrode beyond the acrylic block to prevent contact between the sample and acrylic block, leaving 4 mm of the electrode exposed. The impedance probe was connected to the potentiostat and mounted on a 3-axis computer-controlled positioning stage, giving location information for each impedance measurement with respect to a registration point on the sample, marked with India ink. This apparatus permits measurements with a 0.5 cm linear pitch, (measured at the center of the impedance probe) resulting in multiple unique measurement locations on each tissue sample. For each measurement, the probe was translated using the 3-axis computer-controlled positioning stage, using the India ink registration point as the origin. The probe was then inserted ~4 mm into the tissue, and the galvanostatic measurements were recorded. During the measurement, the sample and EIS probe were enclosed in a Faraday cage made of fine copper mesh (not shown in figure 1 for image clarity) to minimize external electrical interference. The methods for using the 4-electrode plunge probe closely follow previous reports (Tsai et al 2000, Haemmerich et al 2009, Laufer et al 2010).

The data in this work are reported both as measured impedance, Z, and equivalent tissue conductivity, σ_{eq}, using a calibrated probe constant, K. The probe constant accounts for the
effective current path through the tissue (area/length), enabling the derivation of conductivity from the measured conductance. The probe constant was found following the methodology reported previously (Haemmerich et al, 2009, Laufer et al, 2010). Briefly, the real portion of the admittance, \( Y \) (and \( Y = 1/Z \)) of NaCl solutions of known concentration, ranging from 0.001 to 0.15 M, were measured and compared to the known and reported solution conductivity (Vanysek, 2013). As ideal solutions do not have an imaginary component to the admittance (Laufer et al, 2010), the appropriate \( K \) values were determined by matching the real part of the measured tissue impedance to the real part of the calibration solution impedance. Furthermore, the temperature of the NaCl solutions was recorded before and after calibration and was determined to vary by <3 °C.

Additionally, if the tissue is approximated as an electrical resistor and capacitor in parallel (Laufer et al, 2010) the capacitive and resistive current paths can be assumed to be the same, especially in the absence of an imaginary component of the measured impedance for the calibration solutions. Therefore, the probe constant found using the real part of the calibration solution may be used in the determination of the equivalent tissue relative permittivity, \( \varepsilon_{r,eq} \), which is also reported here. Specifically, using the previously reported method (Laufer et al, 2010), \( Y = G + j\omega C \), where \( G \) is the conductance, \( C \) is the capacitance of tissue, \( j \) is the imaginary number \( \sqrt{-1} \), and \( \omega \) is the angular frequency. Calibration allows separation of \( G \) and \( C \) using the expressions, \( G = K\sigma_0 \); \( C = K_{\varepsilon_0}\varepsilon_{r,eq} \) for determination of the equivalent tissue properties (Gabriel et al, 2009, Laufer et al, 2010) with \( \varepsilon_0 \) being the permittivity of free space.

Measurements were also performed specifically to determine the random error. For one sample, 13 distinct locations in normal tissue were each measured three consecutive times with the probe remaining inserted in the specimen. The average measurement error between the three consecutive measurements was found to be 0.53% across the entire frequency range. The overall data reported presents the arithmetic mean of all measured locations for the specific tissue type (i.e. normal tissue or tumor tissue) across all 10 patients, with the error bars representing the standard deviation from the mean. Additionally, data from a single representative case is also presented to show the variation within a sample as compared to variation.
across all samples. Therefore, the data presented show the mean and a measure of dispersion about that mean, as displayed by the error bars of one standard deviation. A single tail student $t$-test was used to compare the mean values for tumor tissue and normal tissue in order to determine statistical significance between mean values of impedance and derived parameters ($\sigma_{eq}$ and $\epsilon_{eq}$).

3. Results and discussion

3.1. Tissue impedance

Figure 2(a) shows the average impedance modulus and phase with the standard deviation for a representative case. It can be seen from figure 2(a) that the normal tissue impedance decreases from $\sim1,980 \pm 432 \Omega$ at 100 Hz to $\sim170 \pm 31 \Omega$ at 1 MHz, while in the same frequency range tumor impedance is observed to decrease from $\sim183 \pm 43 \Omega$ to $\sim107 \pm 15 \Omega$. Despite the magnitude of variance (varying between 21%–18% for normal tissue, and 23%–14% for tumor tissue over the frequency range from 100 Hz to 1 MHz) the impedance modulus and phase were both found to be statistically significant between normal and tumor tissue at all frequencies ($p < 0.01$) for this representative case. The data in figure 2 comprises 27 distinct locations in normal tissue and 12 distinct locations in tumor tissue from a representative case.

Next, the data from all samples across all patients is presented for the 132 measurements. Figure 3 shows the average impedance modulus and phase with the standard deviation from the mean for all measurement points across the 10 patients for both, normal and tumor tissues. From figure 3(a), it can be seen that the magnitude of normal tissue impedance remains higher than tumor tissue impedance across the entire frequency range (100 Hz–1 MHz), varying from $\sim2 \text{ k}\Omega$ at 100 Hz to $\sim225 \Omega$ at 1 MHz, while tumor impedance ranges from $290 \Omega$ at 100 Hz to $107 \pm 15 \Omega$ at 1 MHz.
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at 1 MHz. Figure 3(b) shows the phase trends as a function of frequency. The normal tissue displays a net capacitive response with a maximum phase shift of 33° at 50 kHz, where the source current leads the measured voltage. By contrast, the tumor tissue data shows a much different behaviour with a maximum phase shift of 10° at ~316 kHz. Furthermore, the phase changes by no more than ~5° from 100 Hz to ~4 kHz with a maximum of 10.1° at 316 kHz, suggesting a largely resistive behaviour across all measured frequencies as opposed to a more capacitive response in the normal tissue.

The reported standard deviation values were found to be much larger than the quantified measurement error. Across the measured frequency range, the impedance modulus had an average variation of 30% for normal tissue and 40% for tumor tissue. Given that measurement error was ~0.5%, the variability in the measured data is attributed to electrode polarization at low frequencies, in-sample heterogeneity, differences in samples sizes, and inherent sample-to-sample differences (Morimoto et al. 1993, Faes et al. 1999, Gabriel et al. 2009, Laufer et al. 2010). Tissue desiccation is also a contributor and has been reported previously (Morimoto et al. 1993, Faes et al. 1999, Jossinet and Schmitt 1999, Haemmerich et al. 2009, Laufer et al. 2010). The effect of tissue drying was quantified, as described in the methods section, and was found to be at most 10% of the mean impedance modulus over the 90 min measurement period. It is also worth noting that the measurements on the representative sample for the liver metastasis from CRC cases had a smaller variation as compared to the variation across all samples, suggesting further support for the previous observation that sample-to-sample differences can significantly contribute to impedance data variability (Laufer et al. 2010). Overall, the reported trends along with the variation in data follow results reported previously.

Figure 4. The plots show average equivalent electrical conductivity, $\sigma_{eq}$, and equivalent relative permittivity, $\varepsilon_{r, eq}$, as obtained by using the salt solution calibration method, described in the methods section. (a) Shows the electrical conductivity for the present work with the standard deviation in the calculated mean. The plot also shows data from the only two other studies (also see table 1) on cancerous human liver tissues. On the data points for previous studies, dotted lines are included to provide a visual guide to observe the trends. $\sigma_{eq}$ values are plotted as a mean for both normal and tumor tissue across all measurements. The mean electrical conductivity between normal and tumor tissue was statistically significant ($p < 0.01$) for all frequencies. (b) The equivalent electrical permittivity is plotted as function of the frequency. The standard deviation in data is not shown explicitly to maintain visual clarity (see table 2 for comparison of data with standard deviations between present work and past reported results). $\varepsilon_{r, eq}$ values are plotted as a mean for both normal and tumor tissue across all measurements with statistical significance ($p < 0.01$) for nearly all frequencies.
3.2. Tissue properties

The probe constant, $K$, was determined in this work by calibrating the impedance probe against a range of NaCl solutions with known properties, as described in the methods section. Using this calibration approach, an equivalent tissue conductivity and permittivity were calculated and are shown in figure 4 with a more complete summary presented in tables 1 and 2. Figure 4(a) shows that the conductivity of normal liver tissue is $0.035 \pm 0.018 \text{ S m}^{-1}$ at 100 Hz and increases monotonically to $0.271 \pm 0.088 \text{ S m}^{-1}$ at 1 MHz, similar to those previously reported for normal human liver tissue (Geddes and Baker 1967, Haemmerich et al. 2003, 2009, Laufer et al. 2010). Electrical conductivity of tumor tissue was $0.252 \pm 0.107 \text{ S m}^{-1}$ at 100 Hz increasing to $0.471 \pm 0.150 \text{ S m}^{-1}$ at 1 MHz with trends once again matching previous reports as shown in figure 4(a).

Table 1 compares $\sigma_{\text{eq}}$, normal to previously reported data for similar tissue types. The values from the present work fall between the only two previously reported studies for cancerous human liver tissue. It can be seen that over the 1 kHz–400 kHz range (Haemmerich et al. 2009) reported values for normal tissue shows an increase in equivalent conductivity from $0.074 \pm 0.020 \text{ S m}^{-1}$ to $0.260 \pm 0.062 \text{ S m}^{-1}$, while Laufer et al. (2010) report an increase from $0.03 \pm 0.01$ to $0.164 \pm 0.03 \text{ S m}^{-1}$ in the same frequency range, demonstrating a variation of $\pm 30\%$ at 1 kHz. The previous report from Laufer et al. (2010) also presents a detailed discussion on the observed variability in their data, citing 40% variance for tumor tissue and highlighting previous studies for animal models with reported variation reaching as high as 53% (Haemmerich et al. 2002). Therefore, the trends for the mean values and subsequent standard deviations reported here for the equivalent electrical conductivity are once again consistent with previous reports.

In order to differentiate between normal and tumor tissue, the mean values for the electrical conductivity were statistically compared and found to be different ($p < 0.01$), despite the variability in the data. The caveat for equivalent electrical properties of tissues derived using a calibrated probe constant based on well-mixed, homogeneous solutions is that the exact current path geometry induced by the probe within a heterogeneous tissue sample is not known a priori. The difference between the calibrated and true geometry likely generates additional variance among measured values, and quantification of the calibration error is beyond the scope of this paper.

Figure 4(b) shows the equivalent relative permittivity, $\varepsilon_{r, \text{eq}}$, of the normal and tumor tissues. Again, the trends reported here match the previously reported trends for equivalent tissue permittivity (Gabriel et al. 1996, Laufer et al. 2010) with a comparison summary presented in table 2 with reported variability as the standard deviation about the mean. In this work, $\varepsilon_{r, \text{eq}}$ for normal tissue decreases from $3.1 \times 10^5 \pm 2.3 \times 10^5$ to $1.8 \times 10^2 \pm 6.6 \times 10^2$ from 100 Hz to 1 MHz, while $\varepsilon_{r, \text{eq}}$ for tumor tissue decreases over the same frequency range from $1.8 \times 10^6 \pm 2.3 \times 10^6$ to $1.4 \times 10^5 \pm 8.5 \times 10^2$. It should be noted that the probe constant used for determination of $\varepsilon_{r, \text{eq}}$ is the same as that used for $\sigma_{\text{eq}}$ and is based on ideal solutions that are believed to not show significant imaginary components (Laufer et al. 2010). However, a potential source of uncertainty that can arise is if the solutions show measurable imaginary components and if the calibration approach does not account for these effects explicitly (Gabriel et al. 1996, Laufer et al. 2010). It is important to point out that unlike previous studies, the difference between the mean permittivity of normal and tumor tissue is found to be statistically significant ($p < 0.01$) in the 100 Hz–800 kHz range, except in the vicinity of the intersection of the two curves (8–20 kHz) shown in figure 4(b). These measurements suggest that electrical permittivity may also be a valuable parameter to characterize tissues over select frequency ranges.
Table 1. A summary of the equivalent electrical conductivity, $\sigma_{eq}$ from this work and recent studies.

<table>
<thead>
<tr>
<th>Frequency Hz$^a$</th>
<th>$\sigma_{eq_normal}$ S m$^{-1}$</th>
<th>$\sigma_{eq_tumor}$ S m$^{-1}$</th>
<th>$\sigma_{eq_normal}$ S m$^{-1}$</th>
<th>$\sigma_{eq_tumor}$ S m$^{-1}$</th>
<th>$\sigma_{eq_normal}$ S m$^{-1}$</th>
<th>$\sigma_{eq_tumor}$ S m$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.035 ± 0.018</td>
<td>0.252 ± 0.107</td>
<td>0.074 ± 0.024</td>
<td>0.425 ± 0.258</td>
<td>Not measured</td>
<td>Not measured</td>
</tr>
<tr>
<td>1000</td>
<td>0.037 ± 0.020</td>
<td>0.267 ± 0.114</td>
<td>0.074 ± 0.020</td>
<td>0.419 ± 0.235</td>
<td>0.030 ± 0.01</td>
<td>0.166 ± 0.08</td>
</tr>
<tr>
<td>10000</td>
<td>0.050 ± 0.022</td>
<td>0.296 ± 0.123</td>
<td>0.092 ± 0.023</td>
<td>0.430 ± 0.228</td>
<td>0.042 ± 0.01</td>
<td>0.181 ± 0.08</td>
</tr>
<tr>
<td>100000</td>
<td>0.109 ± 0.034</td>
<td>0.347 ± 0.126</td>
<td>0.179 ± 0.041</td>
<td>0.461 ± 0.202</td>
<td>0.091 ± 0.02</td>
<td>0.222 ± 0.07</td>
</tr>
<tr>
<td>400000</td>
<td>0.190 ± 0.056</td>
<td>0.399 ± 0.130</td>
<td>0.260 ± 0.062$^b$</td>
<td>0.504 ± 0.191</td>
<td>0.164 ± 0.03</td>
<td>0.272 ± 0.07</td>
</tr>
<tr>
<td>1000000</td>
<td>0.255 ± 0.078</td>
<td>0.450 ± 0.142</td>
<td>0.288 ± 0.082</td>
<td>0.535 ± 0.188</td>
<td>Not measured</td>
<td>Not measured</td>
</tr>
</tbody>
</table>

$^a$ As the measurement frequencies vary significantly across reported data, the nearest decade was chosen for comparison. The 400kHz data point was chosen as it is a common value for RF ablation.

$^b$ The closest match for Haemmerich et al. data to 400kHz data was 460kHz.
Table 2. A summary of the equivalent relative electrical permittivity, $\varepsilon_{r, eq}$ from this work and recent studies.

<table>
<thead>
<tr>
<th>Frequency Hz$^a$</th>
<th>$\varepsilon_{r, eq\text{ normal}}$</th>
<th>$\varepsilon_{r, eq\text{ tumor}}$</th>
<th>Haemmerich et al (2009)</th>
<th>Laufer et al (2010)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>$3.1 \times 10^3 \pm 2.3 \times 10^3$</td>
<td>$1.8 \times 10^6 \pm 2.3 \times 10^6$</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>1000</td>
<td>$1.1 \times 10^4 \pm 3.8 \times 10^4$</td>
<td>$3.1 \times 10^5 \pm 1.6 \times 10^5$</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>10000</td>
<td>$4.7 \times 10^4 \pm 1.5 \times 10^4$</td>
<td>$5.0 \times 10^5 \pm 1.5 \times 10^5$</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>100000</td>
<td>$1.3 \times 10^5 \pm 3.8 \times 10^4$</td>
<td>$9.4 \times 10^5 \pm 3.8 \times 10^5$</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>400000</td>
<td>$4.5 \times 10^3 \pm 1.1 \times 10^3$</td>
<td>$3.2 \times 10^5 \pm 1.6 \times 10^5$</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>1000000</td>
<td>$1.8 \times 10^1 \pm 6.6 \times 10^2$</td>
<td>$1.4 \times 10^7 \pm 8.5 \times 10^2$</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

$^a$ As the measurement frequencies vary significantly across reported data, the nearest decade was chosen for comparison. The 400kHz data point was chosen as it is a common value for RF ablation.
3.3. Theoretical validation

A theoretical analysis for biological tissues uses the Cole–Cole fit, a dispersion model reported explicitly in 1941 (Cole and Cole 1941); however, origins of the use of this model can be traced back to 1928 (Cole 1980) when Fricke proposed an equivalent circuit model for the impedance of red blood cells with a semi-circular locus in the complex plane, referred to as a Nyquist plot in other fields (Bard and Faulkner 1980). The main value of the Cole–Cole fit for biological media lies in the fact that the Cole–Cole fit allows for the reduction of experimental complex impedance data to four fitting parameters: \( \rho_\infty \), the resistivity as the frequency approaches infinity, \( \rho_0 \), the resistivity as the frequency approaches zero, \( f_c \), the characteristic frequency, and \( \alpha \), the fractional power representing the depression of the semi-circular arc from the real axis. The inverse of two resistivity parameters are also commonly reported as conductivities (\( \sigma_\infty, \sigma_0 \)), and the characteristic frequency can also be reported as its inverse, the time constant, \( \tau_c \). For the range of frequencies probed in this study, the Cole–Cole fit is given by the form given in equation (1) as previously reported (Cole and Cole 1941, Jossinet and Schmitt 1999) for tissue resistivity, including fitted parameters for cancerous human liver tissue (Laufer et al 2010).

\[
\rho^* = \rho_\infty + \frac{\rho_0 - \rho_\infty}{1 + \left(j\frac{f}{f_c}\right)^\alpha}
\] (1)

Figure 5. The plot shows Cole–Cole fits to the experimental mean resistivity for normal and tumor tissue. The error in the Cole–Cole fits was less than 1% at any frequency and the adjusted \( R^2 \) value for both fits was greater than 0.99. The characteristic parameter for the Cole–Cole fits was determined to be \( \alpha = 0.65 \) for normal tissue, and \( \alpha = 0.26 \) for tumor tissue. The characteristic frequencies, \( f_c \) were found to be 8.836 Hz for normal tissue and 506.737 Hz for tumor tissue respectively. The corresponding resistivity values were found to be, \( \rho_\infty, \text{normal} = 2.70 \Omega \text{m}, \rho_0, \text{normal} = 29.40 \Omega \text{m} \) for normal tissue, and \( \rho_\infty, \text{tumor} = 0.26 \Omega \text{m}, \rho_0, \text{tumor} = 4.36 \Omega \text{m} \) for tumor tissue.
Figure 5 shows the normal and tumor tissue Cole–Cole fits to equation (1). The error in the fits was less than 1% at all frequencies and the adjusted $R^2$ value for both fits (normal and tumor tissue) was greater than 0.99. For normal tissue, $\alpha$ was found to be 0.65, and 0.26 for tumor tissue. The characteristic frequencies, $f_c$ were found to be 8.836 Hz for normal tissue and 506.737 Hz for tumor tissue. The corresponding resistivity values were found to be, $\rho_\infty$, normal = 2.70 $\Omega$ m, $\rho_0$, normal = 29.40 $\Omega$ m for normal tissue, and $\rho_\infty$, tumor = 0.26 $\Omega$ m, $\rho_0$, tumor = 4.36 $\Omega$ m for tumor tissue. For tissues in the probing frequency range of 100Hz–1 MHz, it is expected that $0 < \alpha < 1$. In a previous study on human hepatic tissue (Laufer et al 2010) the Cole–Cole parameter was reported as $\alpha = 0.50$ for tumor tissue and $\alpha = 0.63$ for normal tissue. In contrast, a previous review article (Foster and Schwan 1989) reported data from a variety of biological tissues and materials to typically have a value for $\alpha$ between 0.3 and 0.5.

The Cole–Cole fits (Cole and Cole 1941, Cole 1980, Foster and Schwan 1989, Laufer et al 2010) are an empirical model, originally developed for dielectric materials with tissue properties reduced to a set of four parameters to generate resistor–capacitor equivalent circuit models as discussed extensively in previous research results and reviews (Cole 1980, Pethig and Kell 1987, Foster and Schwan 1989, Laufer et al 2010). The two key parameters are the characteristic frequency $f_c$, and the Cole–Cole parameter $\alpha$ (also referred to as the distribution parameter). The value for $f_c$ represents the cut-off frequency for an RC circuit, and $\tau_c = 1/f_c$ is characteristic time representing the time taken for the capacitor of magnitude $C$ to charge through a resistor $R$ to 63.2% of the steady-state value. As the probing frequencies in this work were below 1 MHz, the frequency range lies within $\alpha$-dispersion (note, the dispersion parameter $\alpha$ is distinct from the distribution or Cole–Cole parameter $\alpha$, though both have the same symbolic notation in published literature) and $\beta$-dispersion range for tissues (Pethig and Kell 1987, Foster and Schwan 1989, Gabriel et al 1996). Consequently, the $f_c$ or $\tau_c$ would ideally represent the characteristic frequency or characteristic time for charge storage on cell membranes (the dominant capacitive component in tissue).

In this study, the parameters for the Cole–Cole fit were found using an iterative manual search, where the quality of the fit is measured by its residual to the experimental data. The fit which produced the smallest residual is presented. For the data collected in this study, the $f_c$ of tumor tissue is greater than that of normal tissue by nearly two orders of magnitude, suggesting that the capacitance of human liver metastases from CRC is much smaller than for normal liver tissue. In other words, the tumor tissue shows a more resistive than capacitive behavior, in-line with the data trends observed in figure 3(b) where the phase shift from an ideal resistor equivalent circuit was significantly smaller for tumor tissue than for normal tissue.

4. Summary and conclusions

In this paper, 132 point-wise ex vivo impedance measurements on liver metastases and the surrounding normal liver tissue from 10 patients with CRC are reported, making it the largest study of its kind to date. The impedance for normal liver tissue is found to decrease from nearly 2 k$\Omega$ at 100Hz to less than 225 $\Omega$ at 1 MHz. The phase of the impedance indicates a net capacitive phase shift, with a maximum phase shift of nearly 33° at 50 kHz. In contrast, tumor tissue showed was significantly more resistive behavior with only a ~10° phase shift over the entire frequency range and a change in impedance modulus of approximately 150 $\Omega$ over the 4 decade frequency range. The reported values for electrical conductivity and permittivity lie between those previously reported. Even though the overall trends in the data reported here match previous trends, the variability in reported conductivity and permittivity values point to the need for standard calibration methods and reliable calibration data. The direct implication
of the measured impedance is that the electrical conductivity of tumor tissue is 2–5 times greater than that of the surrounding normal tissue over the measured frequency range. The difference in permittivity for normal and tumor tissues is also found to be statistically significant ($p < 0.01$) over nearly all measured frequencies. The Cole–Cole fits of normal and tumor tissue demonstrate the significantly higher characteristic frequency of tumor tissue in contrast to normal liver tissue, consistent with previously reported impedance measurements.

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