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### Impedance-based diagnostic of liver steatosis

**Damià Parramon**, **Ivan Erill**, **Anton Guimerà**, **Antoni Ivorra**, **Ángeles Muñoz**, **Anna Sola**, **Constantino Fondevila**, **Juan Carlos García-Valdecasas** and **Rosa Villa**

1 Biomedical Applications Group, Centro Nacional de Microelectrónica, 08193 Bellaterra, Spain
2 Department of Bioengineering, University of California at Berkeley, Berkeley, CA 94720, USA
3 Department of Experimental Pathology, IIBB-CSIC, IDIBAPS, Roselló, 161, 7a Planta, 08036 Barcelona, Spain
4 Liver Transplant Unit, Digestive Disease Institute, Hospital Clinic, University of Barcelona, Spain

Email: Damia.Parramon@cnm.es

**Abstract**

Hepatic steatosis is a widespread condition of high prevalence in Western populations, and its asymptomatic nature represents a hefty problem in liver surgery and transplantation. Current diagnostic methods rely mainly on biopsy and blood tests, and are thus time-consuming and expensive. Here we report the use of direct impedance measurements on liver tissue as a promising alternative to conventional diagnostic methods in surgery and transplantation. Working on a dual Zucker Fat (ZF), Zucker Lean (ZL) rat experimental model, we show that certain parameters extracted from multi-frequency impedance measurements correlate well with the presence of steatosis and that these results can be adequately approximated with bi-frequency measurements extracting the impedance modulus at 1 kHz and the impedance phase angle at 5.7 kHz. We further support our findings on a theoretical model of tissue impedance, and the simulations carried out suggest a possible mechanism to expound the negative effect of steatosis in post-transplant graft function.

**Keywords:** liver steatosis, *in vivo* monitoring, electrical impedance
1. Introduction

Hepatic steatosis, commonly known as fatty liver, is a relatively common condition defined by an excessive accumulation of fat in hepatocytes and typically associated to several factors, such as alcohol, diabetes, excess body fat or insulin resistance (Bellentani et al. 2000, Browning et al. 2004). Even though it is usually asymptomatic, hepatic steatosis can lead to severe steatohepatitis (liver inflammation) that, if unchecked, may often develop into cirrhosis. Moreover, hepatic steatosis has been shown to exert a considerable influence on liver surgery and liver transplantation success rates. In liver surgery, steatotic livers have been described to affect postoperative recovery, patient outcome and mortality. Even in its mildest form, hepatic steatosis significantly increases the risk of postoperative complications and reduces the degree of insults a liver may withstand during the surgical procedure (Vetelainen et al. 2007). Similarly, hepatic steatosis has also been linked to impoverished graft function and postoperative recovery of donors in living donor liver transplantation (Imber et al. 2002, Angulo 2006).

When present, hepatic steatosis poses a delicate problem for liver surgery, as it may prioritize certain surgery methods, contraindicate surgery for mild liver conditions, or even call for postponing surgery until steatosis has been rightly addressed by other means (Vetelainen et al. 2007). The predicament is all the more acute in transplantation, where hepatic steatosis increases the risk of graft non-function and generally affects liver performance after orthotopic liver transplantation, thus introducing the notion of rejecting organs with high levels of steatosis (Koneru et al. 2002, Angulo 2006). Unfortunately, the asymptomatic nature of most hepatic steatosis cases makes detection extremely difficult. Fatty livers are not only asymptomatic from a patient standpoint, but they tend also to be relatively asymptomatic in blood tests, although they have been linked to sporadic elevated serum aminotransferase, phosphatase and gamma-glutamyltranspeptidase activity (Tekin et al. 2004). Moreover, and since there is a considerable number of risk factors for hepatic steatosis, it is also difficult to predict steatosis based solely on patient history, and current guidelines recommend liver biopsy, together with measurement of glutamyl-transpeptidase and alanine aminotransferase, to diagnose steatosis (Bellentani et al. 2000, McCullough 2004, Levitsky et al. 2004, Rubbia-Brandt and Hadengue 2005). Liver biopsy, however, is not an adequate solution for the diagnosis of steatosis in donor grafts. Besides being invasive, biopsy imposes a substantial delay that cannot be afforded in such cases, and it still carries a potential sampling error and a large associated cost (Maharaj et al. 1986, Fiorini et al. 2004).

Not surprisingly, the risks associated with hepatic steatosis and the limitations of biopsies to diagnose it have spurred research into alternative methods for estimating liver steatosis. The most studied of these methods is blood test-based enzymatic prediction, which relies mostly on alanine transaminase,
aspartate transaminase and gamma-glutamyl-transferase levels and is often complemented by other
predictors, like gender, age, body-mass index (BMI) or waist circumference, leading to reference
indexes of hepatic steatosis (e.g. Fatty Liver Index and SteatoTest; Poynard et al. 2005, Bedogni et al.
2006). In spite of their reported efficacy, enzymatic methods still require time-consuming blood tests
and thus are not suitable for transplantation. Besides enzymatic prediction, however, only a handful
of other non-invasive, faster methods have been proposed to date. On the one hand, imaging
techniques such as quantitative X-ray computed tomography (Iwasaki et al. 2004) and
ultrasonography (Fontana and Lok 2002, Schneider et al. 2005) have been suggested as predicting
methods for steatosis, yet their efficiency as predictors is relatively low, interpretation of the results
is somewhat subjective and the methods often fail to distinguish between steatosis and other related
conditions (Yao et al. 2001, Hepburn et al. 2005, Schneider et al. 2005). On the other hand, and
based on the established fact that hepatic steatosis reduces tissue perfusion in human liver grafts
(Seifalian et al. 1998), laser Duplex–Doppler flowmetry methods have also been proposed for direct
evaluation of steatosis in liver allografts, but their efficacy has yet to be thoroughly established.
In this work, we present a new minimally-invasive method to estimate hepatic esteatosis in liver
allografts based on local and direct bioimpedance measurements of liver tissue. Our results on the rat
liver model show that direct liver impedance measurement is an accurate predictor of hepatic
steatosis, and we substantiate this finding with simulations of the effects of intracellular fat intrusion
on impedance in a 2D theoretical model of tissue impedance.

2. Materials and methods

2.1. Impedance sensor and measuring equipment
To conduct precise 4-electrode electrical impedance measurements of tissue, a custom needle-
electrode array was manufactured using a rectangular slab of methacrilate as the array base. The slab
was perforated with a mini-drill and, after alignment to ensure matching needle lengths, four 3 mm-
long Ø 0.5 mm gold needles were glued to the drilled holes (figure 1). Needles were then welded to
connecting wires on the back side of the methacrilate slab, and the bonding area was generously
coated with silicone to effectively insulate it. The inner voltage sensing needles were situated 7.62
mm apart over the slab longitudinal axis, and set at 2.54 mm from each outer needle. This
configuration generated a four-point electrode with an experimentally validated 1.7 cm cell constant.
To perform 4-electrode multi-frequency bioimpedance measurements on rat livers, a custom-made,
portable instrumentation system and its accompanying software were developed as described
previously (Gómez et al. 2006). The system injects an AC current into the sample through the outer
electrodes and measures differentially the resulting potential across the inner electrodes. The
instrumentation carries out a serial frequency sweep of 4-electrode measurements at 30 different frequencies logarithmically spaced between 100 Hz and 100 kHz within the β dispersion zone (Schwan 1957).

![Sensor photo](image)

![Sensor diagram](image)

**Figure 1.** (A) Sensor photo (B) Sensor diagram detailing the disposition of the needle electrodes on the methacrilate slab for 4-electrode measurements.

2.2. Rat liver model

To validate bioimpedance as a marker for hepatic steatosis, the study was conducted on two genetically different rat models with disparate propensities to fat accumulation in the liver: Zucker Lean (ZL) and Zucker Fat (ZF) male rats (Amersi et al. 2003, Koteish and Mae 2001). Zucker Fat rats inherit obesity as an autosomal recessive trait, resulting in obese, hyperphagic, insulin resistant and hyperlipidemic adults. Both kinds of rats were maintained on a standard fat chow diet and water *ad libitum* and housed in a room with a 12-12 h light-dark cycle and an ambient temperature of 22 °C. Both ZL and ZF rats were fed for either 13 or 14 weeks to obtain different degrees of hepatic steatosis. A total of 8 rats were divided into two groups of 4, each containing 2 ZL and 2 ZF rats, which were sacrificed on week 13 and 14, respectively. Table 1 summarizes the main physiological parameters measured in both groups.

2.3. Surgical techniques and experimental procedure

Before starting the surgical procedure, rats were weighted and their height measured from nates to hind legs. After inducing inhalational anesthesia with Isoflurane gas, the abdomen was shaved and opened with a midline incision, thus exposing the liver and other abdominal organs. After surface
cleansing, the needle-electrode array was manually positioned over one of the three liver lobes and
the electrodes were then inserted into liver tissue by applying a slight pressure. Five multi-frequency
impedance measures were taken in each of the three liver lobes (right (A), center (B) and left (C)).
After completing the impedance measurements, rats were sacrificed and the liver was extracted and
weighted. Thereafter, 5 mm-thick samples were taken from each lobe and fixed in formaldehyde to
be embedded in paraffin for histological evaluation. All the above procedures were conducted under
the supervision of our institutions’ Research Commission and strictly adhered to EU guidelines for
the handling and care of laboratory animals.

Table 1. Most relevant physiological parameters for both experimental rat groups. The steatosis level for
each lobe (right (A), center (B) and left (C)) is shown in percentage and was estimated by histological
analysis.

<table>
<thead>
<tr>
<th>Rat</th>
<th>Weight (g)</th>
<th>Length (cm)</th>
<th>Liver Weight (g)</th>
<th>Age (weeks)</th>
<th>Steatosis level</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZL1</td>
<td>340</td>
<td>23.5</td>
<td>11.74</td>
<td>13</td>
<td>&lt;5 &lt;5 0</td>
</tr>
<tr>
<td>ZL2</td>
<td>329</td>
<td>23.5</td>
<td>11.93</td>
<td>13</td>
<td>&lt;5 0 0</td>
</tr>
<tr>
<td>ZF1</td>
<td>426</td>
<td>23</td>
<td>21.46</td>
<td>13</td>
<td>45 30 40</td>
</tr>
<tr>
<td>ZF2</td>
<td>419</td>
<td>23</td>
<td>26.15</td>
<td>13</td>
<td>70 75 80</td>
</tr>
<tr>
<td>ZL3</td>
<td>356</td>
<td>23.5</td>
<td>13.20</td>
<td>14</td>
<td>&lt;5 &lt;5 0</td>
</tr>
<tr>
<td>ZL4</td>
<td>382</td>
<td>24</td>
<td>14.30</td>
<td>14</td>
<td>5-10 15-20 5</td>
</tr>
<tr>
<td>ZF3</td>
<td>394</td>
<td>23</td>
<td>21.46</td>
<td>14</td>
<td>70 60 70</td>
</tr>
<tr>
<td>ZF4</td>
<td>462</td>
<td>24</td>
<td>25.58</td>
<td>14</td>
<td>55 70 60</td>
</tr>
</tbody>
</table>

2.3. Histological analysis

One sample was collected from each liver lobe, fixed in 10% formalin and embedded in paraffin to
preserve the tissue architecture. Additional lobe samples were snap frozen in liquid nitrogen and
stored at –80°C for future analysis. Fixed liver samples were then sectioned into 4 µm tissue sections,
stained with haematoxylin-eosin and examined on a transmission electron microscope.
Histopathological examination was performed blindly using light microscopy in 30 high-power fields
per sample with a magnification of 40x. Hepatic steatosis was evaluated using a quantitative analysis
based on the number of hepatocytes with fatty changes per field. The principal histologic feature was
the presence of micro- and macrovesicular fatty change in hepatocytes with displacement of the

2.4. Simulations

Measurements of electrical impedance of liver tissues with variable degrees of steatosis were
simulated using previously developed software for bioimpedance simulation (Ivorra et al. 2005,
Ivorra et al. 2005a). The BioZsim bioimpedance simulator generates SPICE netlists (Al-Hashimi 1995) that represent a slice of living tissue, described as a two-dimensional mesh of passive electric components that depend on numerical parameters, such as the plasm (extra-cellular medium) and cytoplasm resistivities, and a two-dimensional map drawn by the user. Each square pixel of the map is thus transformed into a set of passive circuit components (resistances and capacitances), with interconnections between them and components of the adjacent pixels. The electrode, plasmic and cytoplasmic elements are modeled as pure resistive media, while plasmic–cytoplasmic interfaces are assumed to correspond to cell membranes and are modeled as parallel capacitance-resistance elements. It has been shown previously that, when large numbers of elements are combined in a complex tissue schematic, the overall system response differs from the simple Fricke–Morse model and approaches the Cole model, thus constituting a good approximation of living tissue (Ivorra et al. 2005).

Simulations were carried out on 236x236 µm² maps (9 µm²/pixel) containing between 36 (24x24 µm²) and 25 (30x30 µm²) cells. Steatosis was simulated by the intrusion of fat vacuoles inside cells, and several possibilities regarding cell volume were taken into account. In model Fat1, fat intrusion was considered as water replacement, maintaining a constant volume for the fatty cell. In contrast, models Fat2 and Fat3 assumed that intracellular water content remains constant with fat intrusion, thereby leading to cell swelling. In the Fat2 model, cell-to-cell distance was maintained constant in spite of cell swelling, whereas in Fat3 this distance was reduced to compensate swelling. Table 2 lists the values for the different parameters used in the simulations. The parameters for each of the passive electric components listed in Table 2 were extracted from the literature as described previously (Ivorra et al. 2005).

3. Results

3.1. In-vivo multi-frequency measurements of liver impedance

The Bode representation of five in vivo impedance measurements at different frequencies for a single liver lobe in two ZL and two ZF rats is shown in figure 2. As it can be readily appreciated, differences in the impedance modulus between ZL and ZF rats are apparent at low frequencies (100 Hz to 100 kHz), in accordance with the degree of steatosis estimated by histological analysis for both groups. At high frequencies (>50 kHz), however, these differences tend to attenuate as impedance modulus values converge for both groups. Conversely, impedance phase values are remarkably similar for ZL and ZF rats at low frequencies (<1 kHz) but show a peak of substantial deviation in the 5-10 kHz range that can be used to complement impedance modulus readings.
Table 2. Simulation parameters for passive electric components of the tissue model and geometric parameters specific to each of the different fat models.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pixel size</td>
<td>9 µm²</td>
</tr>
<tr>
<td>Plasm resistivity</td>
<td>100 Ω·cm</td>
</tr>
<tr>
<td>Cytoplasm resistivity</td>
<td>100 Ω·cm</td>
</tr>
<tr>
<td>Fat resistivity</td>
<td>100 kΩ·cm</td>
</tr>
<tr>
<td>Membrane capacitance</td>
<td>1 µF/cm²</td>
</tr>
<tr>
<td>Membrane resistance</td>
<td>10 GΩ·cm²</td>
</tr>
</tbody>
</table>

Specific parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Fat 1</th>
<th>Fat 2</th>
<th>Fat 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cells</td>
<td>36</td>
<td>36</td>
<td>25</td>
<td>36</td>
</tr>
<tr>
<td>Cell-to-cell distance</td>
<td>9 µm</td>
<td>9 µm</td>
<td>9 µm</td>
<td>3 µm</td>
</tr>
<tr>
<td>Intracellular water content</td>
<td>constant</td>
<td>variable</td>
<td>constant</td>
<td>constant</td>
</tr>
<tr>
<td>Intracellular size</td>
<td>576 µm²</td>
<td>252 µm²</td>
<td>576 µm²</td>
<td>576 µm²</td>
</tr>
<tr>
<td>Fat size</td>
<td>0 µm²</td>
<td>324 µm²</td>
<td>324 µm²</td>
<td>324 µm²</td>
</tr>
<tr>
<td>Total Cell size</td>
<td>576 µm²</td>
<td>576 µm²</td>
<td>900 µm²</td>
<td>900 µm²</td>
</tr>
</tbody>
</table>

To obtain additional, more informative parameters for predicting steatosis, the Nyquist representation of these same results, which charts the imaginary and real components of impedance, was plotted and analyzed. As it can be seen in figure 3, the difference between ZL and ZF rats can be neatly observed in the Nyquist representation, as the plotted impedance spectra implicitly integrate impedance phase and modulus information in a single graph. From the Nyquist representation, three different parameters corresponding to the Cole-Cole model of bioimpedance were extracted (Cole 1932, Grimnes and Martinsen 2000). Values for the center frequency ($f_c$), the relaxation or characteristic frequency for the tissue corresponding to the maximal value of the impedance imaginary component for each rat, and the estimated high- and low-limit resistances ($R_0$ and $R_\infty$) are shown in figure 4. As it can be observed, $R_0$ and $f_c$ values tend to discriminate well between ZL and ZF rats, while $R_\infty$ values, as expected from the converging values of impedance modulus at high-frequencies, fail to distinguish between both groups. $R_0$ and $f_c$ values show also complementary resolutions on steatosis levels: while $R_0$ values seem to discriminate better among elevated degrees of steatosis, $f_c$ values tend to differentiate better between low steatosis levels.
Figure 2. Bode plot for (A) impedance modulus and (B) impedance phase readings, and (C) stained tissue snapshots at x20 magnification corresponding to a single lobe in 13- and 14-week old ZF and ZL rats. Figure labels indicate rat type (ZL/ZF), age (13/14 weeks) and the percentage of steatosis as derived from histological analysis.

Figure 3. Nyquist representation for the same impedance readings as in figure 2. Figure labels indicate rat type (ZL/ZF), age (13/14 weeks) and the percentage of steatosis as derived from histological analysis. The error bars for the real and imaginary components of impedance are shown for indication at some of the measured frequencies. $R_\infty$, $R_0$ and $f_c$ values can be estimated geometrically from the Nyquist representation.
Figure 4. Representation of the three parameters ($R_0$, $R_\infty$, and $f_c$) derived from the Cole-Cole model in all the rats and lobes measured in this study. Figure labels indicate the percentage of steatosis as derived from histological analysis, while x-axis labels denote rat type (ZL/ZF), age (13/14 weeks) and lobe (A, B, or C). Boxes define the 1st and 3rd quartiles for a batch of measurements in a single lobe, while bars denote the highest and lowest values measured in each lobe.

3.2. Tissue multi-frequency impedance simulation results.

The Bode and Nyquist representations of simulation results for the different fat intrusion models described in figure 5 are shown in figure 6 and figure 7. As it can be seen in figure 6, the simple intrusion of fat vacuoles (Fat1 model) does not result a significant increase of impedance modulus at low frequencies (100 Hz to 1 kHz). Instead, it is the decrease in total extracellular space due to cell swelling which leads to a moderate increase in low-frequency impedance modulus for the Fat2 model, in which there are fewer cells and thus less extracellular space, and to a drastic increase when cell-to-cell distance is decreased to accommodate swelling (Fat 3 model). At high frequencies, the impedance modulus raises comparably in all three fat models, indicating that the simple effect of fat
intrusion should be in principle detectable at high frequencies. Impedance phase angle results do also
differentiate between normal tissue and those with reduced extracellular space, with a noticeable
deveiation at low and mid frequencies (100Hz to 10 kHz). The combined effect of impedance modulus
and phase angle variations between fat intrusion models can be observed in the Nyquist plot shown in
figure 7, with a proportional increase in $R_0$ with decreasing extracellular space, and a positive shift in
$R_\infty$ due to simple fat intrusion. The huge reduction in extracellular space and the consequent increase
in impedance modulus of the Fat3 model is reflected by a significant enlargement of the imaginary
component of impedance in this model.

Figure 5. Graphical description of the simulation models. Control corresponds to the normal cell
without fat vacuoles, while Fat1 refers to a cell with intruded fat vacuoles but no cell volume increase.
Fat2 and Fat3 correspond to cells with intruded fat vacuoles and constant cytoplasm water content,
which leads to cell swelling. In the Fat2 model, the increase in cell size does not lead to a decrease in
cell-to-cell distance (i.e. extracellular space), whereas this is taken into account in the Fat3 model.
Cell membrane is not explicitly drawn, but rather modeled as the transition between intra- and extracellular media.
Figure 6. Bode plot for impedance modulus (A) and impedance phase angle (B) for simulation results in the four fat intrusion models.

Figure 7. Nyquist representation of impedance for simulation results in the four fat intrusion models.
3.3. Discrimination of steatosis with bi-frequency data

In the light of the aforementioned results of multi-frequency impedance measurements, and since comprehensive multi-frequency analyses are difficult to implement and automate in a clinical setting, a bi-frequency model was introduced to evaluate the feasibility of discriminating between steatotic and normal livers on two frequency data. Based on the observed values of Cole-Cole parameters ($f_c$, $R_0$), impedance modulus at 1 kHz ($R_{1kHz}$) and impedance phase angle at 5.7 kHz ($\theta_{5.7kHz}$) were proposed as an approximation of $R_0$ and an estimator of phase variation at $f_c$, respectively. The results summarized in figure 8 reveal that both parameters are able to competently discriminate between normal and steatotic livers, thus providing the means to develop a simpler steatosis predictor for clinical use.

Figure 8. Values for (A) impedance modulus at 1 kHz ($R_{1kHz}$) and (B) impedance phase angle at 5.7 kHz ($\theta_{5.7kHz}$) in all the lobes and rats measured in this study. Labels indicate the percentage of fat as estimated from histological analysis. The x-axis labels denote rat type (ZL/ZF), rat number (1-4) and lobe (A-C). Shaded areas indicate linear threshold discrimination between normal and steatotic rat lobes.

4. Discussion

The results presented above demonstrate that direct impedance measurements can be a solid indicator of steatosis in liver, as they systematically discern between ZL and ZF rat groups. Even though the correlation between measured impedance and specific steatosis levels as determined by histological analysis is not extremely dependable across the full range of steatosis levels, the ability of impedance measurements to thoroughly discriminate between ZL and ZF groups suggests that the method is reliable and that further experimental data points might be able to adjust a non-linear correlation between both variables. Furthermore, it must be taken into account that some histological results may
be skewed by local variations in tissue structure and subjective appreciation (Fiorini et al. 2004). In this sense, and even though further experimental results are required, the fact that impedance is also able to discern systematically between 13- and 14-week old ZF rats, which should on average have a similar degree of steatosis, suggests that impedance, being a broader measure, could be a more robust estimator of liver steatosis.

Even though the ability of impedance to discriminate steatosis levels did not come as a surprise, the mechanisms by which it does so had not been anticipated, as they differ from the standard methods for inferring fat content using impedance. Estimation of body fat through impedance measurements (Roubenoff et al. 1995) to determine the body-mass index, which is a routine procedure today, works by indirectly assessing the percentage of body water and, thereafter, the relative amount of fat (Lukaski, 1989). However, impedance-based estimation of fat percentage in a tissue model is notably different from the indirect impedance assessments of body fat used in the determination of BMI, and a tissue-based model is required to interpret these measurements. Before carrying out the experimental measurements, the leading hypothesis was that intracellular fat intrusion would lead to a marked increase in the impedance modulus at high frequencies. Tissue can be modeled as a network of interconnected elements in which each cell is seen as a parallel resistance-capacitor component (cell membrane) in series with a plasmatic resistance and in parallel with an extracellular resistance (Ivorra et al. 2005). The extracellular medium is sensed mostly by low frequency measurements, since low frequency currents cannot penetrate the cell membrane, while high frequency measurements have been shown to correlate mostly with cell membrane and the plasmatic medium (Grimnes and Martinsen 2000). Consequently, it was assumed that fat intrusion in the plasmatic medium would lead to an increase in the impedance modulus at high frequencies, leaving it relatively intact at low frequencies. The experimental data reported here, however, explicitly contradict this hypothesis, showing the most significant changes between ZL and ZR rats at low and mid frequencies (100 Hz to 100 kHz). It is in this respect that the simulations carried out on several possible models of fat intrusion shed some light into the mechanism underlying steatosis detection through direct impedance measurements.

A straight comparison of simulation results between models in which cell volume is either held constant or augmented in proportion to the size of the fat vacuole reveals that cell swelling due to fat intrusion, instead of the simple presence of fat within the cell, is the major contributor to impedance change in steatotic tissue. Swelling reduces the available extracellular space and therefore results in the observed increase in impedance modulus at low frequencies, which is even more acute when the extracellular volume is assumed to shrink due to swelling. Simulation results do show also that, as expected, the presence of fat vacuoles generates an increase in impedance modulus at high frequencies. However, the magnitude of this rise is not as significant as that observed at low
frequencies, and it is also more difficult to appreciate in the experimental results due to the lower
signal-to-noise ratio available at high frequencies. Impedance phase angle results also support this
hypothesis. The apparent differences in phase angle at mid frequencies observed both in simulations
and experimental data are also suggestive of cell swelling and a reduction of the extracellular space,
which changes the proportion of cell membrane traversed by the injected current, thereby inducing
changes in phase angle.

These findings on the underlying model of impedance-based diagnosis of steatosis are relevant
because they contribute to explaining the reported influence of steatosis in post-transplant graft
function. Previous experiments have shown that ischemia in explanted grafts can be detected through
impedance at low frequencies and that this detection is mainly due to cell swelling (Grimnes and
Martinsen 2000, Gomez et al. 2006). In this respect, prior swelling due to steatosis can have dramatic
effects on the capacity of tissues to withstand ischemic insults. On the one hand, the presence of
steatosis-induced swelling prior to ischemia is suggestive of compromised micro-vascularization,
thus probably aggravating the effects of sustained anoxia due to a poorer diffusion of oxygen through
the tissue. On the other hand, a noticeable degree of swelling prior to the onset of ischemia also
results in a more limited capacity of cells to swell in response to the stopping of active ion pumps
induced by ischemia, leading to increased ischemia-induced necrosis and apoptosis in the tissue.
Overall, both factors can significantly contribute to explain the increasing incidence of Primary Non-
Function (PNF) in severely steatotic grafts (Seifalian et al. 1999).

The successful evaluation of a bi-frequency model for hepatic steatosis detection is also significant in
the context of the ultimate application of such a system. Even though the full multi-frequency
analysis of bioimpedance is more precise, detection of steatosis using a bi-frequency model relaxes
the hardware and software requirements of a commercial system. Since the proposed system is
intended for clinical use and implies direct contact with living tissue, it must comply with stern safety
regulations. In this respect, a decrease in hardware and software requirements can make such a
system more competitive with conventional alternatives, such as indices of enzyme levels.

5. Conclusion
In this work, we report for the first time anywhere the detection of hepatic steatosis through direct
impedance measurements of liver tissue. We show that some parameters ($f_c$ and $R_0$) obtained through
a multi-frequency analysis of impedance correlate well with histological assessment of steatosis and
that these parameters can be adequately approximated with real impedance values from just two
different frequencies (1 and 5.7 kHz). We also propose and validate through simulation a tissue
model of impedance that explains the mechanism of steatosis-mediated variations in impedance
readings and suggests how steatosis influences post-transplant graft function. These findings are
significant because they introduce for the first time a correlation between steatosis and measured electrical impedance based on a reliable theoretical frame and pave the way for the development of devices for the rapid diagnosis of steatosis in the clinical setting.

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