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Use of Electrical Impedance Spectroscopy to Distinguish Cancer from Normal Tissues with a Four Electrode Terminal Setup

Abstract: Cancer and normal tissues are visually different from each other, especially so in more advanced cancer stages. More important, they are not only visually contrasting, but if an electric field is applied to both tissue types and the frequency is varied in a wide range, it will be seen that the two tissue types in general have a spectral response divergent from each other and this has to do with the characteristics of cancer tissues in contrast to normal ones. In this work, Electrical Impedance Spectroscopy is applied to try to distinguish cancer from healthy tissues by means of their impedance spectrum using a four-electrode-terminal setup. The use of the four-terminal-setup setup is important to circumvent the impact of electrode polarization at frequencies below 1 kHz.

Keywords: Cancer cells, electrical impedance spectroscopy, four electrode terminal setup, malignant tumors, normal cells, normal tissues, impedance spectrum.

https://doi.org/10.1515/cdbme-2020-3088

1 Introduction

Electrical impedance spectroscopy (EIS) is a technique that can possibly be used to distinguish cancer from normal tissues based on the measurement of the electric properties of tissues when submitted to frequency variable electric fields [1–8]. It consists on the application of a small amplitude voltage or current signal and the measurement of the biological tissue response [1]. It is label-free in the sense that no staining is needed and it also does not modify the tissue structure. Very small voltages and currents should be applied in order not to degrade tissue vitality. It is already used in several fields and is currently under development to distinguish cancer cells and tissues from normal ones [1–9].

The interesting aspect about applying EIS to cancer tissues is that they show a spectral response quite different from their normal counterparts and this has all to do with the effects of the cancer growing within a normal tissue environment. Also, important information can be derived from their spectral profile. Cancers in general have larger water and salt content when compared to normal organs, caused by leaky blood vessels and the lack of a properly working lymphatic system [10–13]. This imbalance in fluid transport leads to the accumulation of interstitial fluid, liquids and salts inside the tumour. For EIS, higher water and salt content translates in higher tissue conductivity, and consequently, lower impedance.

2 Experimental Procedure

The severe combined immunodeficient (SCID) mouse was purchased from Charles River. The autosomal recessive scid mutation leads to the absence of functional B and T lymphocytes, therefore the human tumour cells can be xenografted onto mice of this strain [14].

A total of 1 million human colon carcinoma cells HT29 (ATCC) were subcutaneously injected into SCID mouse in the area of the right scapula. The animal was kept in an individual ventilated cage under pathogen-free conditions, fed with sterile standard food and water ad libitum, and was regularly monitored concerning subcutaneous the tumour growth. When the tumour reached 1 cm^3, the animal was sacrificed, the tumour and the organs excised and their impedance measured ex-vivo. Measurements were performed immediately after sacrifice. Organs and primary tumour were placed in phosphate buffer saline (PBS) for the measurements. An array of four needle electrodes was used to measure the impedance and provide a four-terminal stimulation. The needles used had a total length of 12 mm and a diameter of 0.4 mm.

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The potentiostat Gamry Interface 1000 was used to perform all experiments. A 14 mV amplitude voltage signal was applied and the frequency was varied from 1 MHz up to 100 mHz in steps of ten points per decade.

All results were processed and plotted in MATLAB.

3 Results

3.1 Spectrum of Mouse Normal Organs

In the brain, both right and left hemispheres were measured in longitudinal and transversal direction. The average of the impedance magnitude and phase for both hemispheres and directions is also shown. Some differences in the measured impedance was observed from longitudinal to transversal directions showing the anisotropy of the brain, while right and left hemispheres did not show significant differences from each other. This anisotropy in the directions is common in biological tissues and is due to the different tissue arrangement from one direction to the other.

In mouse kidneys, the degree of anisotropy observed experimentally between transversal and longitudinal directions was larger than for the brain (see Figure 2). Furthermore, the right kidney showed a lower impedance than the left one. One possible explanation can be that one kidney (right) was full of liquids with salts which decreased its impedance.

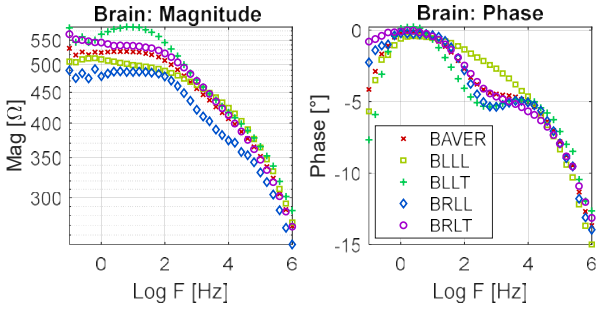

Figure 1: SCID mouse brain impedance magnitude and phase, measured in both hemispheres (left and right) and in longitudinal and transversal directions. Legend: BAVER (brain average), BLLL (brain left hemisphere longitudinal), BLLT (brain left hemisphere transversal), BRLL (brain right hemisphere longitudinal) and BRLT (brain right hemisphere transversal).

The mouse liver was measured in longitudinal and transversal directions (Figure 4). Figure 5 show the results from the impedance measurements from the thigh muscle of one leg of the mouse. The muscle in general has a high salt and water content, therefore it shows a smaller impedance when compared to other organs.

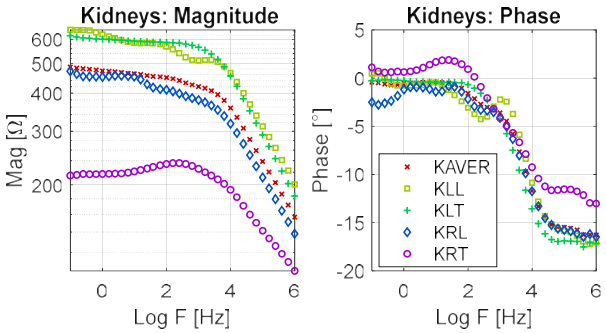

Figure 2: SCID mouse kidney impedance measured in right and left side kidneys, in longitudinal and transversal directions. The average (aver) is plotted in red. Legend: KAVER (kidneys average), KLL (kidney left longitudinal), KLT (kidney left transversal), KRL (kidney right longitudinal) and KRT (kidney right transversal).

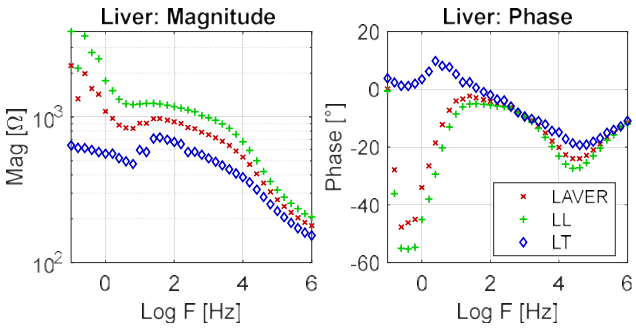

Figure 4: Mouse liver impedance measured in longitudinal and transversal directions. Legend: LAVER (liver average), LL (liver longitudinal) and LT (liver transversal).

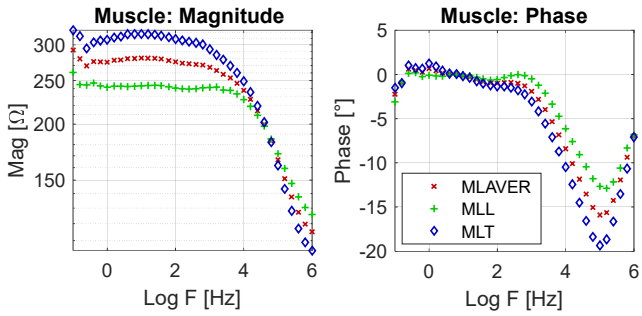

Figure 5: Mouse thigh muscle leg impedance measured in longitudinal and transversal directions. Legend entries: MLAVER (muscle leg average), MLL (muscle leg longitudinal), MLT (muscle leg transversal).

The mouse heart was measured in both longitudinal and transversal directions, which also showed a significant anisotropy in the impedance measured between both directions (Figure 6). The lungs had a large impedance presumably due to their high air content when compared to other organs.

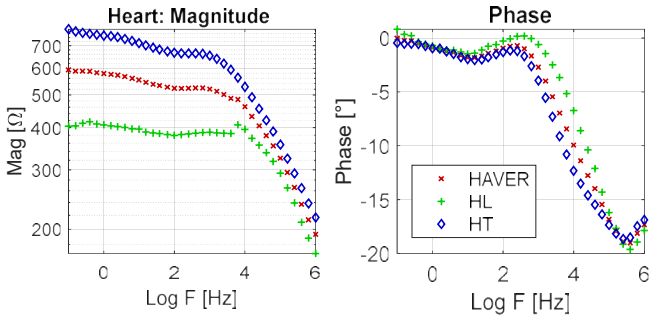

Figure 6: Mouse heart impedance. Legend: HAVER (heart average), HL (heart longitudinal) and HT (heart transversal).

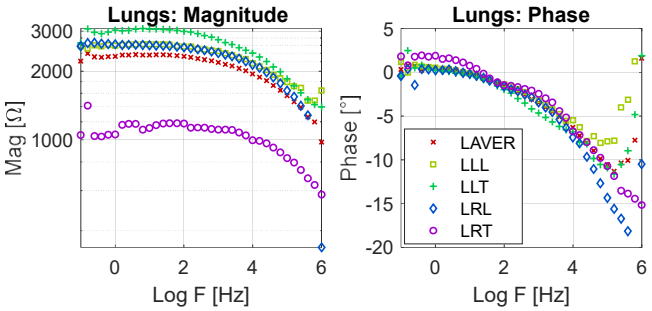

Figure 7: Lungs impedance. Legend: LLAVER (lungs average), LLL (left lung longitudinal), LLT (left lung transversal), LRL (lung right longitudinal) and LRT (lung right transversal).

3.2 Tumor vs. Normal Tissues Spectrum

As cancer can form distant metastases in almost any organ it is of interest to plot impedance magnitude and phase of the primary tumour nodule with other organs (Figures 8-10). The primary tumour nodules had the lowest impedance from all measured organs or equivalently the highest conductivity. In principle, the same results were observed in another study with two different tumour types and mice implanted with UT-SCC-5 (tongue squamous cell carcinoma) and UM-SCC-10A (laryngeal squamous cell carcinoma), using a two-electrode-terminal setup [1]. An explanation for such behavior is the accumulation of interstitial fluid inside the tumours due to leaky blood and mal-functioning lymphatic vessels [10–13]. Higher water and salt content, which are major ingredients of the interstitial fluid, will cause a decrease in the measured impedance magnitude of the tumour when compared to normal organs. Equivalently, as the conductivity is derived from the real part of the impedance magnitude, a lower magnitude will translate into a higher conductivity.

Interesting to note is that the impedance values correlate closely to organ structure, water and salt content (Figures 8 – 10). Organs with high water and salt content, e.g. muscle, showed a lower impedance magnitude or equivalently higher conductivity. Conversely, organs e.g. lungs with low liquid content presented the lowest conductivity, equivalent the highest impedance of all measured organs.

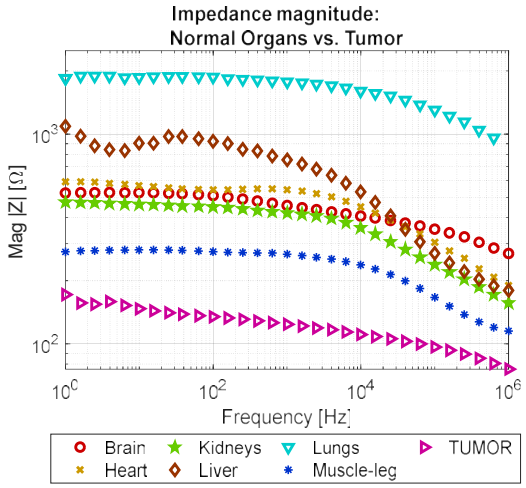

Figure 8: Impedance magnitude of mouse organs plotted together with tumour. All values are averages.

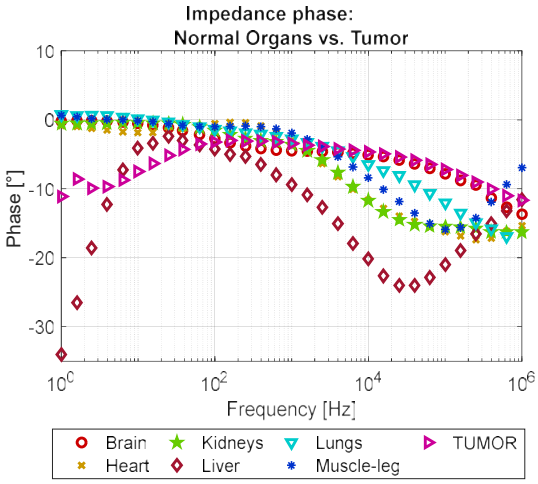

Figure 9: Impedance phase of mouse organs and tumor.

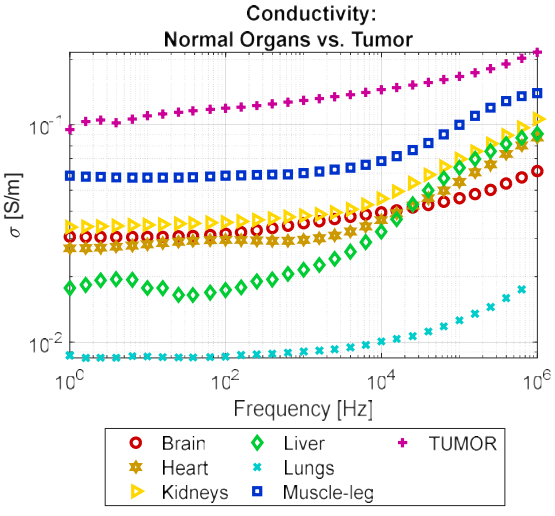

Figure 10: Conductivity of mouse normal organs and primary tumour nodule. Tumours showed the highest conductivity among all measured organs.

Table 1: Electrical Parameters Mouse Normal Tissues & Primary Tumor

Organ	Mag |Z| [Ω]			Phase |Z| [°]		
	1kHz	10kHz	100kHz	1kHz	10kHz	100kHz
Lung	1868	1773	1301	0	-3	-13
Liver	905	754	270	-4	-9	-21
Heart	568	543	305	-2	-3	-16
Brain	525	456	352	0	-4	-8
Kidney	462	420	239	0	-3	-15
Muscle	280	267	166	0	-2	-16
Tumor	147	124	97	-7	-3	-7

Table 1 cont.

Organ	σ [mS/m]		
	1kHz	10kHz	100kHz
Lung	8	9	12
Liver	18	21	64
Heart	28	30	55
Brain	30	35	46
Kidney	35	38	70
Muscle	57	60	100
Tumor	110	130	170

Table 1 shows that the conductivity of the tumour is more than ten times higher than the lung conductivity, and approximately twice higher as the conductivity of the mouse thigh muscle, which is also an organ rich in water and salt content. The brain, the kidneys and the heart have similar values, with small differences while liver showed also small conductivity values, therefore higher impedance values.

4 Conclusion

The results of this experiment showed that the distinction between tumour and normal tissues may be possible by means of Electrical Impedance Spectroscopy. In a previous experiment [1], we obtained similar results using a two-electrode-terminal setup and three other mice, one healthy and two implanted with UT-SCC-5 cell line (tongue squamous cell carcinoma) and UM-SCC-10A (laryngeal squamous cell carcinoma). In that case the two tumours also showed the lowest impedance among all compared organs. In a large set of experiments recently performed where we measured the same tumour type (human prostate cancer PC-3 cells) in five different mice, we observed the same tendency of the tumours to have either the smallest impedance or to be amongst the smallest together with mouse thigh muscle legs (own unpublished results). This bring us to expect that there is a tendency of tumours to have smaller impedance than healthy organs due to their internal composition: high water and salt content, loose connection between adjacent cancer cells, amongst other factors.

Author Statement

Research funding: this research has been developed partially with funding from Forschungszentrum Medizintechnik Hamburg (FMTHH) through project grant 02fmthh2018. Mrs. Viviane Teixeira thanks ProExzellenzia 4.0 program for providing the scholarship to continue her research. Conflict of interest: Authors state no conflict of interest. Ethical approval: animal experiments was approved by the local committee (Behörde für Gesundheit und Verbraucherschutz, Amt für Verbraucherschutz, Lebensmittelsicherheit und Veterinärwesen, assigned project No. N08/2018), supervised by the institutional animal welfare officer and performed in accordance with relevant guidelines and regulations.

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