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In vivo bioimpedance measurement of healthy and ischaemic rat brain: implications for stroke imaging using electrical impedance tomography

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Abstract

In order to facilitate the imaging of haemorrhagic and ischaemic stroke using frequency difference electrical impedance tomography (EIT), impedance measurements of normal and ischaemic brain, and clotted blood during haemorrhage, were gathered using a four-terminal technique in an *in vivo* animal model, a first for ischaemic measurements. Differences of 5–10% in impedance were seen between the frequency spectrums of healthy and ischaemic brain, over the frequency range 0–3 kHz, while the spectrum of blood was predominately uniform. The implications of imaging blood/ischaemia in the brain using electrical impedance tomography are discussed, supporting the notion that it will be possible to differentiate stroke from haemorrhage.

Keywords: electrical impedance tomography, bioimpedance, medical imaging

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

1. Introduction

In order to aid in the rapid diagnosis and treatment of ischaemic stroke, a method for differentiation between ischaemic and haemorrhagic stroke is required. We are currently developing a technique for imaging using electrical impendance tomography (EIT). To aid this, a study was

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undertaken to examine the conductivity spectra of the tissues involved—normal or ischaemic brain and blood, in an *in vivo* setting.

1.1. Bioimpedance

Bioimpedance measurements reflect the dielectric properties of body tissues. Biological tissues typically have three regions of dispersion: α, β and γ (Gabriel *et al* 2009). Low-frequency α-dispersion occurs below 1 kHz and is driven by interfacial polarization phenomena such as the flow of ions across cell membranes, and interaction between fluid and electrodes. The α dispersion is associated with three factors: (1) a frequency-dependent conductance of the protein channels in the cell's membrane. (2) A frequency-dependent counter-ion environment near the charged cell surface. (3) An endoplasmic-reticulum effect in muscle tissue only. The mid-range β dispersion is typically seen around several hundred kHz. The β dispersion is of particular significance and arises from the electrical polarization of material in an electric field. It is mainly due to the polarisation of cellular plasma membranes, which act as barriers to the flow of ions between the intra and extra cellular space. The plasma membrane capacitance, the cell radius and the fluid conductivities determine the associated relaxation time. An additional contribution to this dispersion is due to polarisation of proteins and other organic macromolecules. High-frequency γ dispersion at 100 MHz and above is contributed to largely by the relaxation of polar water molecules. For a review of the relevant literature, see Horesh (2006).

1.2. Methods to measure impedance of a sample: two-terminal versus four-terminal method

There are a number of commonly used techniques for bioimpedance measurements. In the two-terminal measurement technique, the same two leads are used to drive currents into the medium and to acquire potential difference measurements. One potential source of error with this approach is that any impedance associated with the electrodes or leads will contribute to the measurement, which can have a significant effect when measuring low impedance, or at low frequencies. While it is possible to correct for this effect if the electrode impedance is known, alternative measurement techniques, which are not affected by electrode impedance, are preferable. Three-terminal measurements are performed with two terminals (input and output) and a guard, which is common to both. This method greatly reduces the effects of electrode and lead impedance, particularly for low frequency measurements, but does not eliminate electrode impedance completely. Finally, tetrapolar (four-terminal) measurements are performed using two electrodes to drive current and two to record voltage. In principle, this method eliminates series impedances and contact-resistance errors. The impedance of current injecting electrodes becomes immaterial and the impedance of electrodes on the recording becomes negligible if the input impedance of the recording amplifiers is sufficiently high. As a result, most instruments now use the four-terminal method to perform low impedance measurements.

1.3. EIT for stroke detection

EIT is an imaging technique in which differences in impedance, in response to a small injected current, are used to produce an image of the internal impedance of an object. Current medical applications of EIT include real time lung monitoring and mammography (Holder 2004). This work is carried out as part of a wider project, focused on expanding the use of EIT to stroke detection in humans.

Absolute imaging using EIT, where a set of measurements are taken at a single point in time, is possible, but measurement artefacts make its clinical implementation impractical. It is more common for the difference between two, or more, data sets to be used for image reconstruction. These data sets can either be measured at different points in time, or at different frequencies, as the impedance of different biological tissues vary over frequency. Time difference imaging of discrete bodies of undesired tissue (tumors, blood clots, etc) is not practical, as it would be necessary to have an equivalent data set of the affected area containing only healthy tissue. However, frequency difference EIT offers a potential solution, as multiple measurements can be taken at a single point in time, across a range of frequencies.

In idealised situations, such as liquid filled tanks, where only two tissue types are present, weighted frequency difference EIT (WFD-EIT) can be performed (Ahn 2011). This assumes a homogeneous background and so, for cases where only two tissues or media are present, WFD-EIT offers a suitable solution. In more complex situations, such as the head, several tissues types are present—skin, skull, grey/white matter; each of which have a different impedance spectrum. In such cases, WFD-EIT is not appropriate and more advanced frequency difference algorithms are required. One approach is the non-linear fraction estimation method (Malone *et al* 2014). With this, images are made of the normalised impedance spectra of component tissues.

It is therefore essential to know the relative change in impedance over the frequency range used, rather than the absolute impedance value. Importantly, for FD-EIT to be successful, the rate of change of each component's impedance with respect to frequency must be different. If the impedance of different tissues changes at the same rate, then it will not be possible to differentiate them.

2. Literature review

A number of existing publications have detailed similar experiments to those carried out in this work. Variations in the methodology between the studies make direct comparisons of the results difficult. In particular, there are differences in the use of *in vivo* and *in vitro* measurements, the animal used, the measurement technique and the state of the brain at the time of measurement. While different frequency ranges are also used, this will not directly impact the analysis, providing comparable frequency ranges are analysed. The following is a brief synthesis of the existing literature. A summary of results is presented in table 1.

2.1. Heathy brain impedance

Ranck (1963) reported changes of 30% up to 5 kHz, using a three-terminal method on *in vivo* rabbit brain. A phase shift, with a maximum value of 7 degrees, over the range 10–50 Hz was also observed. Logothetis *et al* (2007) observed a 25% change over the same range, but no phase shift.

2.2. Ischaemic brain impedance

Two existing studies are available in the literature that investigates the impedance of ischaemic brain. However, they are not directly comparable to each other due to differing experimental methods. Gabriel *et al* (1996) made *in vitro* measurements in bovine grey and white matter, up to 2 h after excision. Impedance varied by several hundred percent over the range 10–5 kHz. A two-terminal method was used, Bedard and Destexhe (2009) analysed the results of Gabriel,

Table 1. Impedance characteristics of relevant tissues in the published literature. Values are compared over as similar a frequency range as possible, the maximum value of which is indicated by the bracketed term in the final column.

Study	Model/ animal	Measurement technique	Frequency range	Brain tissue type	% Change in impedance
Ranck 1963	*In vivo*, rabbit	three-terminal	5 Hz–50 kHz	healthy	30% (5 kHz)
Gabriel 1996/2009	*In vitro*, bovine	two-terminal	10 Hz–20 GHz	ischaemic	>200% (5 kHz)
Logothetis *et al* 2007	*In vivo*, monkey	four-terminal	10 Hz–5 kHz	healthy	25% (5 kHz)
Wu *et al* 2003	*In vivo*, rabbit	two-terminal	0.1 Hz–1 MHz	healthy and ischaemic	N/A
This work	*In vivo*, rat	four-terminal	10 Hz–3 kHz	healthy and ischaemic	healthy: 40% ischaemic: 30% (3 kHz)

and suggested that the use of this two-terminal measurement technique contributed to the increased changes seen over frequency. The second comparable study was by Wu (2003). The impedance of rabbit brain was measured *in vivo* under normal and ischaemic conditions using a two-terminal method. They observed a difference in impedance between normal and ischaemic brain at lower frequencies (75% difference) than at higher frequencies (15%) difference. It is not clear if the authors corrected for the electrode impedance in their analysis.

2.3. Haemorrhagic stroke: clotted blood impedance

Blood coagulation is a complicated and dynamic physiological process. The red blood cells aggregation is a normal, reversible, physiological process occurring in whole blood. The aggregation may increase blood viscosity, increase the interaction of leukocytes with the endothelium and promote blood coagulation. As the coagulation mechanism is induced, thrombin acts as an enzyme to convert fibrinogen into fibrin fibres that enmesh blood cell and plasma. The blood then becomes a solid gel and clot forms that comprises a meshwork of fibrin fibres running in all directions that entrap erythrocytes and plasma.

Most studies, like Ur (1970), compared the relative change of impedance between whole blood and clotted blood at a single frequency but not over a frequency spectrum. Other studies have concentrated only on heparinised blood, which have shown that the impedance spectrum is flat up to ~2 MHz. Recently, Lei *et al* (2013) measured impedance of whole blood and clotted blood over frequencies using a microfluidic chip. A 10 μL sample of porcine blood was loaded into the well of a microchip. Blood was collected from the local slaughterhouse and mixed with 15% acid citrate dextrose anticoagulation solution. The whole blood was centrifuged and the resulting red blood cells were rinsed. Blood coagulation was induced by adding 0.5 M $CaCl_2$ solution into the blood sample. Blood clot impedance varied around $10^6\,\Omega$ while whole blood impedance was stable at 10^3 across frequencies from 200 to 10 000 Hz.

3. Purpose

This work was undertaken as part of a feasibility study to distinguish ischaemic from haemorrhagic stroke using frequency difference EIT. How the impedance changes over frequency is

of importance for EIT frequency difference imaging. The purpose of this work was to record and compare the impedance characteristics of the principal tissues in the head affected in acute stroke: normal brain, ischaemic brain and clotted blood, as well as a control sample consisting of saline 0.9%. The specific questions to be addressed were:

(a) How does the impedance of the tissues involved change over frequency?
(b) Is there an optimal range over which to measure for FD-EIT?
(c) Are the observed changes large enough for FD-EIT to be successful?

The electronic hardware used for imaging in stroke was developed for imaging fast neural activity (Aristovich 2014) and has high precision in the low frequency band up to 3 kHz. Tissue impedance was therefore recorded in the anesthetised rat, in the range 1 Hz–3 kHz, using this system with a four-terminal method.

4. Methods

4.1. Experimental procedure

4.1.1. In vivo: healthy rat brain. The rats were obtained from Biological Services at University College London and were bred on site. The rats were housed in a room on a 12 h:12 h light:dark cycle with 18–20 air changes per hour and had *ad libitum* access to food and water. All work done with the rats was approved under UK Home Office regulations and was carried out in accordance with the Animals (Scientific Procedures) Act 1986 regulations and the European Directive 2010/63/EU on the protection of animals used for scientific purposes. Female Sprague–Dawley adult rats weighing 300 to 450 g were used for recordings and anaesthesia was induced with 5% isoflurane in 100% oxygen. Rats were intubated and mechanical ventilation was introduced using a Harvard Apparatus Inspira Ventilator (Harvard Apparatus, Ltd, UK). Anaesthesia was maintained with 2% isoflurane in a mixture of oxygen and air throughout the experiment. An artery line was placed in the femoral artery to monitor blood pressure and the femoral vein was also cannulated to allow intravenous access and saline injected as fluid replacement. The body temperature was controlled with a homoeothermic heating unit comprising a heating blanket in which the rat was wrapped and a rectal probe which provided temperature feedback to the system (Harvard Apparatus, Kent, UK). The rat was fixed in a stereotactic frame in the prone position using ear bars. The rat's head was then shaved and the skin incised using a scalpel. A craniotomy 4 mm wide by 7 mm long was drilled on the rat skull. The dura was removed and the 30 channels planar electrode was slid under the skull to insure secure and stable position on the cortical surface. Recordings were made in four rats, using the same protocol as for saline measurements.

4.1.2. In vivo: ischaemic rat brain. Ischaemia was triggered in two out of the four animals, by the four-vessel occlusion method described by Todd *et al* (1986). Both vertebral arteries were permanently occluded by diathermy at the level of the first cervical vertebra, while both carotid arteries were temporarily clamped for 10 min. Recordings were made at the end of this period.

4.1.3. In vitro clotted blood. At the end of the *in vivo* experiments, the animals were euthanized and exsanguination performed. At this point, blood was collected from the intra-arterial line in two rats. It was stored at 4 °C and allowed to clot spontaneously. The clotted blood formed a gel which was inserted into a 50 × 10 mm and a 30 × 10 mm Perspex tube with stainless steel electrodes at both ends. The impedance was measured in two terminal mode,

and the effect of electrode impedance was accounted for by subtraction of the results from the two sets of recordings. Conductivity was measured by using a HP 4284A impedance analyser and the spectrum across frequencies from 20 Hz to 1 MHz was recorded, using constant voltage at room temperature of 18–22 °C.

4.1.4. Saline 0.9%. The 30-channel planar electrode array, and ground electrode, were immersed in a beaker, with diameter 10 cm and depth 20 cm, filled with 0.9% saline solution, at room temperature. Saline recordings were taken as a control before each experiment.

4.2. EIT hardware, planar electrode and current injections

Impedance measurements were made using an EIT system constructed in our laboratory (Dowrick *et al* 2014) comprised of a Keithley 6221 current source for current injection, a BioSemi EEG system for voltage measurement and a custom switch network which enabled the current to be addressed to any chosen pair of the 30 electrodes. A 30 channel planar electrode made from stainless steel foil and silicone rubber was used to record voltages from the rat cortex (Schuettler *et al* 2005). Electrodes were platinised, 0.6 mm in diameter and spaced in a tetrahedral pattern with centres 1.2 mm apart. The electrode array was either immersed in saline solution, for control measurements, or directly positioned on the rat brain after a craniotomy had been performed. An Ag/AgCl reference electrode was placed on the brain at the caudal end of the craniotomy. Current was injected through opposing electrodes at the top left and bottom right of the array, 6 mm apart, while voltage measurements were taken relative to the reference electrode. Measurements were made in steps of 5 Hz from 1 Hz to 100 Hz, steps of 10 Hz from 100 Hz to 990 Hz and steps of 50 Hz from 1000–3000 Hz. At least 50 periods of the waveform were recorded at each frequency. The current amplitude had a peak value of 100 μA in saline and 50 μA on the rat brain. With two electrodes used for current injection, there were 28 remaining electrodes, which yielded a four-terminal measurement when taken relative to the reference electrode. As the voltage at the injecting electrodes was also recorded, this was used to generate two-terminal measurements, for comparison with the four-terminal results. Potential hysteresis effects were investigated, by varying the order of frequencies (sequential increase from 1 Hz, sequential decrease from 3 kHz and random ordering), but no changes were seen in the recorded data greater than 0.01%. This arrangement allowed for simultaneous measurement of 28 voltages at 300 frequencies, in <3 min.

4.3. Data processing

For each set of recordings, the voltage amplitude at each electrode, for each frequency, was extracted from the output of the EEG recording system by filtering around the measurement frequency, demodulating and averaging across the total number of cycles. The nature of the *in vivo* experimental setup means that it is difficult to accurately measure the volume of brain tissue through which current travels. As such, calculating an absolute value for resistivity/conductivity is not practical, but the relative changes in these parameters over frequency can be investigated, as they are directly proportional to changes in the observed voltages.

Analysis of the phase shift over the frequency range used for this study (<3 kHz) showed a maximum shift of less than half of one degree. As such, phase effects were deemed to be negligible, and results are presented as the real part of the complex impedance.

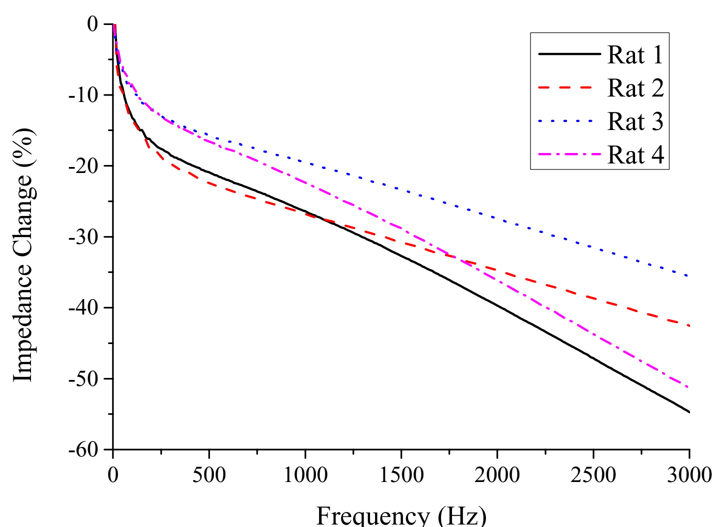

Figure 1. Impedance change of healthy brain. Each line is the mean of the 28 measurements taken in each rat, corresponding to the 28 electrodes used. Only the real component of the impedance is considered, as there are no phase effects over this frequency range.

5. Results

Figure 1 shows the averaged change in impedance of healthy brain for each rat ($n = 28$), taken relative to the impedance at 1 Hz. There is a steep, non-linear decrease in impedance, between 15 and 25%, up to ~250 Hz. Above this frequency, there is a near linear decrease in the impedance of between 15 and 40%. Figure 2 shows all of the measurements taken, for a single rat, for both healthy and ischaemic brain. The maximum standard deviation is 9%, at 400 Hz, for healthy brain and 8%, at 3 kHz, for ischaemic brain. One-way ANOVA was used at each frequency to compare the data for healthy and ischaemic brain, yielding $p < 0.05$ below 500 Hz and $p < 0.1$ over the rest of the range, indicating that two sets of data are significantly different. Figure 3 directly compares the mean impedance changes of healthy ($n = 112$, four rats) and ischaemic brain ($n = 56$, two rats), alongside that of saline ($n = 116$) and blood ($n = 2$). The error bars indicate one standard error. Measurements in saline demonstrate that there was no significant impedance change greater than noise of 0.01%. Over the range used. Healthy *in vivo* rat brain showed a non-linear decrease of 15% in impedance over 0–250 Hz while ischaemic brain displayed a smaller decrease of impedance, 7%, with a more linear slope over the same frequency range. Above 250 Hz, the impedance of both brain types reduces at the same rate. An additional change of approximately 30% is seen over the remainder of the frequency range. Blood showed a 10% change between 0 and 250 Hz, with negligible changes at higher frequencies. The advantages of the four terminal measurement technique over the two terminal approach have previously been discussed. The impedance change was calculated for both techniques (figure 4). When using two terminals, the magnitude of the impedance change at low frequencies is 10–20% larger than for the four terminal method, and the gradient becomes shallower. This illustrates the importance of eliminating the effect of electrode impedance from the measurement.

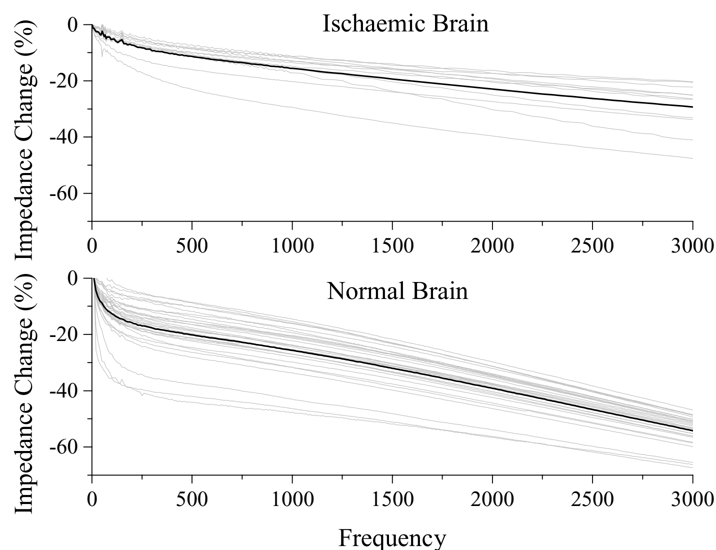

Figure 2. All measurements ($n = 28$) of normal and ischaemic brain for a single rat. The mean values are denoted by the black line.

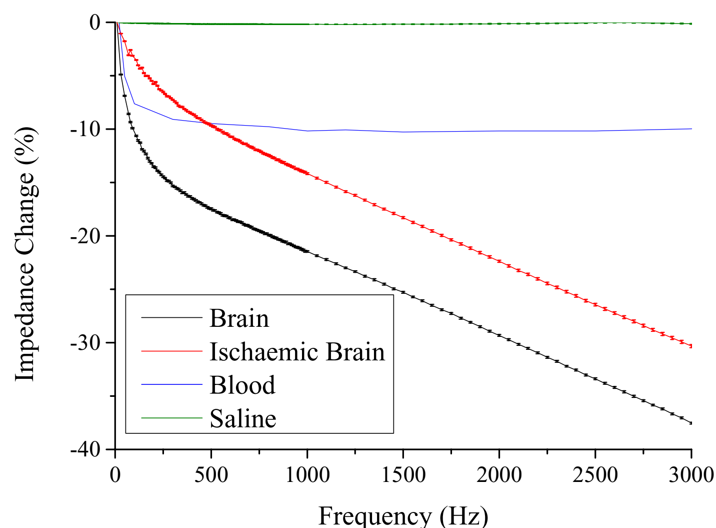

Figure 3. Comparison of impedance of normal brain, ischaemic brain and blood. Mean values, calculated using all measurements, are shown. The error bars, corresponding to the standard error, are shown for saline, healthy and ischaemic brain data. $n = 112$ (four rats) for brain, $n = 56$ (two rats) for ischaemia, $n = 56$ for saline, $n = 2$ (two rats) for blood.

6. Discussion

In general, good agreement can be seen between our present results and the material previously published (table 1). The reported change in impedance of healthy brain, ~40%, is comparable to the values of 30 and 25% reported by Ranck (1963) and Logothetis *et al* (2007). In terms of the measured phase shift, the data reported by Logothetis *et al* (2007)

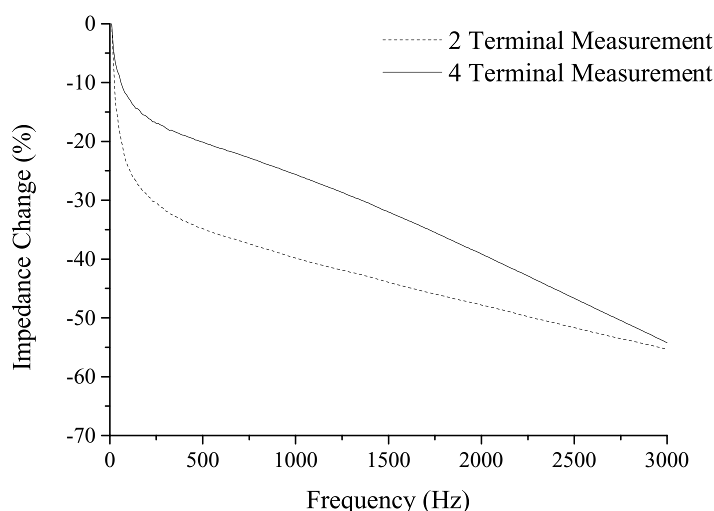

Figure 4. Comparison of average impedance changes for two ($n = 8$) and four ($n = 112$) terminal measurements. The four terminal measurements give a more accurate result, as it ignores the impedance of the injecting electrodes.

supports those reported here, where a negligible phase shift was found over the frequency range considered.

We report in this work that the change in ischaemic brain is less than that of healthy brain, particularly at low frequencies, with a maximum change of ~30%. Ranck (1963) reported changes of up to 200% in ischaemic tissue, but this was measured *in vitro* several hours after death, which will have affected the impedance of the tissue. We also report that the difference seen between ischaemic and healthy brain is greatest at lower frequencies, which is in agreement with Wu *et al* (2003).

For the measurements of blood impedance, our results largely agree with the existing literature on the topic, with constant impedance seen above a few hundred Hz. There are, however, some differences when analysing the lower frequency range. The results reported by Lei (2013) suggest that the impedance of clotted blood is constant across frequency. Our results suggest that there is some change of about 10% at frequencies below 250 Hz. The most plausible explanation for the differences is the different frequency ranges used. Lei (2013) did not record below 200 Hz and did not report any significant change. This was probably because the majority of the changes occur in the lower frequencies. Additionally, there is a difference in the method used for clotting of the blood, which may impact on the results. Our clotted blood sample was obtained by a spontaneous coagulation process, as opposed to adding $CaCl_2$ solution. The physiological mechanism by which the impedance of blood changes at frequencies <250 Hz is not fully understood and without further investigation, any explanation would be speculative in nature. However, in terms of the implications for FD-EIT, it is acceptable to consider the results from a purely phenomenological point of view.

This work was undertaken as a feasibility study to distinguish ischaemic from haemorrhagic stroke using frequency difference EIT. Our *in vivo* measurements show a clear difference of impedance between the three different constituents: healthy brain, ischaemic brain and blood. The noticeable contrast in tissue impedances suggest that frequency difference imaging of stroke will be possible across these frequencies. For distinguishing between blood and normal brain, the contrast between the two tissues became larger as frequency was increased.

This implies that FD-EIT of haemorrhage should be performed over as wide a frequency range as possible. For ischaemic brain tissue, imaging in the range 500 Hz–3 kHz using FD-EIT would not be possible, as the impedance changes at the same rate as healthy brain tissue. In theory, it should be possible to use the frequency range below 500 Hz for imaging, as the two tissue types have different response over this range. This will present a greater challenge than the imaging of haemorrhage, as the changes involved are substantially smaller. It is therefore plausible to conclude that while both are possible, detection of haemorrhagic stroke will be easier than ischaemic stroke, as larger contrasts in tissue impedance will aid the imaging procedure.

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