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**A Novel Microwave Sensor to Detect Specific Biomarkers in Human Cerebrospinal Fluid and Their Relationship to Cellular Ischemia During Thoracoabdominal Aortic Aneurysm Repair**

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### Article

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# Journal of Medical Systems

## A novel microwave sensor to detect specific biomarkers in human cerebrospinal fluid and their relationship to cellular ischemia during thoracoabdominal aortic aneurysm repair

--Manuscript Draft--

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| <b>Abstract:</b>                                     | Thoraco-abdominal aneurysms (TAAA) represents a particularly lethal vascular disease that without surgical repair carries a dismal prognosis. However, there is an inherent risk from surgical repair of spinal cord ischaemia that can result in paraplegia. One method of reducing this risk is cerebrospinal fluid (CSF) drainage. We believe that the CSF contains clinically significant biomarkers that can indicate impending spinal cord ischaemia. This work therefore presents a novel measurement method for proteins, namely albumin, as a precursor to further work in this area. The work uses an interdigitated electrode (IDE) sensor and shows that it is capable of detecting various concentrations of albumin (from 0 to 100 g/L) with a high degree of repeatability at 200 MHz ( $R^2 = 0.991$ ) and 4 GHz ( $R^2 = 0.975$ ). |

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6 **A novel microwave sensor to detect specific biomarkers in human cerebrospinal fluid**  
7 **and their relationship to cellular ischemia during thoracoabdominal aortic aneurysm**  
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## Abstract

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3 Thoraco-abdominal aneurysms (TAAA) represents a particularly lethal vascular disease that  
4 without surgical repair carries a dismal prognosis. However, there is an inherent risk from  
5 surgical repair of spinal cord ischaemia that can result in paraplegia. One method of reducing  
6 this risk is cerebrospinal fluid (CSF) drainage. We believe that the CSF contains clinically  
7 significant biomarkers that can indicate impending spinal cord ischaemia. This work  
8 therefore presents a novel measurement method for proteins, namely albumin, as a precursor  
9 to further work in this area. The work uses an interdigitated electrode (IDE) sensor and  
10 shows that it is capable of detecting various concentrations of albumin (from 0 to 100 g/L)  
11 with a high degree of repeatability at 200 MHz ( $R^2 = 0.991$ ) and 4 GHz ( $R^2 = 0.975$ ).  
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## Introduction

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26 Thoraco-abdominal aneurysms (TAAA) represent a particularly lethal vascular disease,  
27 resulting from the continuous dilation of any part of the descending aorta from the left  
28 subclavian artery to the bifurcation of the aorta into the iliac branches in the abdomen.  
29 Ultimately, the natural history of any aortic aneurysm is the inherent risk of dissection or  
30 rupture. Without treatment TAAAs are associated with a particularly dismal prognosis, with 5  
31 year survival in non-operated patients as low as 10% [1-3].  
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38 Elective surgical repair for TAAAs, when successful can provide survival rates that are  
39 comparable to that of the general population [4]. However, TAAA open surgical or  
40 endovascular aneurysm repair (EVAR) is major extensive surgery and carries a significant  
41 risk of mortality and post-operative complications of which the most feared and devastating  
42 is that of paraplegia. This is a direct consequence of the restriction of spinal cord blood flow  
43 during surgical repair and consequently causes spinal cord ischaemia (SCI) [5]. Published  
44 rates of spinal cord ischaemia are varied worldwide can have been reported as high as 30%  
45 [6-10].  
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Over the past half century numerous adjuncts to reduce the risk of SCI have been developed and are routinely employed to provide neuro-protection through the maintenance of adequate perfusion to the spinal cord. These methods include:

- Deep hypothermic circulatory arrest which decreases the body's metabolic activity and oxygen demand [11-13] .
- Motor-evoked potentials (MEP) which is able to provide near real time assessment of spinal cord function intraoperatively through stimulating the motor cortex and recording any subsequent muscle action potentials and potentially alert the surgeon of impending SCI [14-16] .
- Sequential clamping and reattachment of patent intercostal arteries to the graft inserted onto the aorta which directly supply the spinal cord with blood [17].
- Cerebrospinal fluid drainage (CSFD) which has a strong evidence basis underpinning its use, reducing the pressure of cerebrospinal fluid (CSF) surrounding the spinal cord and consequently increases spinal cord perfusion [2, 18-19] .

Researchers at Liverpool Heart and Chest Hospital and Liverpool John Moores University believe that the drained CSF may harbour clinically important information that can provide indications to SCI. With this in mind the authors are developing a sensor that can detect in real time changes in clinically relevant biomarkers in the CSF that correlate to impending spinal cord ischaemia and hence providing a tool that alerts the surgeon to intervene.

Our current research is evolving around developing a sensor that utilises electromagnetic (EM) waves, particularly microwaves. The authors have already demonstrated the ability of this technology to detect various substances including, glucose at physiological levels, lactate, and oils [20-24]. Furthermore, they have demonstrated the possibility to detect lactate in water, phosphate buffered solution, and CSF. Lactate is commonly used as a marker of tissue hypoxia and hence lactate CSF levels have previously been researched with respects to its correlation to spinal cord ischaemia. However, although there is a good correlation to spinal cord ischaemia and rises in CSF lactate, there is a lag between its rise and development of CSF lactate [25]. There is now considerable interest with respects to specific biomarkers that can be biochemically analysed and are more sensitive to spinal cord ischaemia than lactate [26-31]. In particular, interest has arisen in the area of protein level monitoring, and so the aim of this work is to determine the ability of EM wave sensors to measure varying quantities of protein.

## Methods

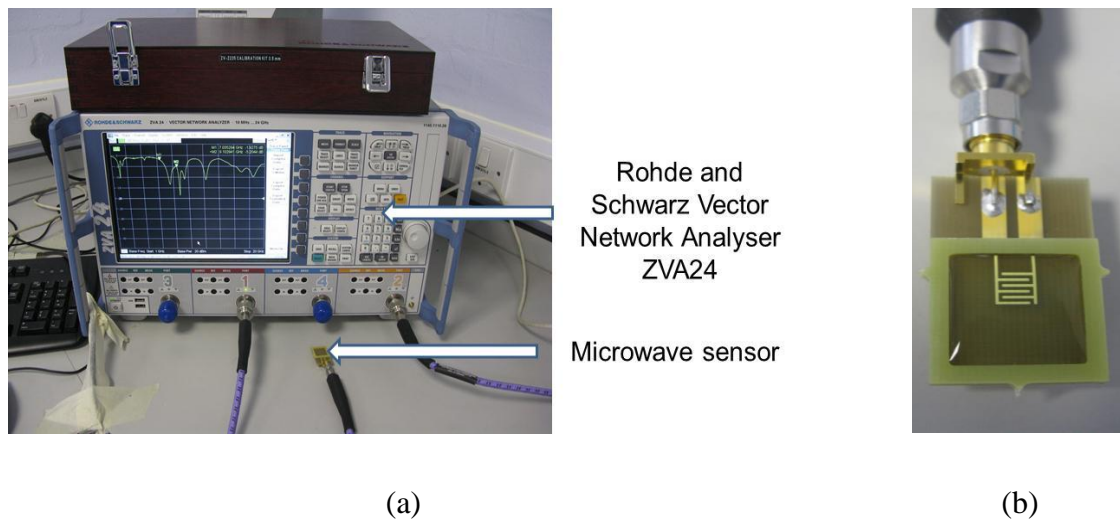
### *Sample preparation*

Bovine serum albumin (A2153, Sigma-Aldrich) was hydrated using deionised water. Dilutions, in g/L, were made as follows: 0, 1, 10, 40 and 100. The samples were prepared and measured in the same day, with a short period allowed for them to acclimatise to room temperature prior to measurement.

### *Acquisition measurements*

Measurement was performed using a ZVA-24 Rohde and Schwarz Vector Network Analyser (VNA). The instrument can generate and measure frequencies up to 24GHz, although for this experimental procedure 15GHz was the maximum frequency attained due to the limitation of the type of connector used on the sensor itself.

Figure 1 depicts the experimental set up with an interdigitated electrode (IDE) connected to the VNA. Initially we found that the sensor is extremely sensitive to both temperature and volume. Therefore, all samples were allowed to confluence to room temperature and repeated measurements (n=10) were taken in quick succession for all dilutions to establish repeatability.



**Figure 1.** Illustrating (a) the Rohde and Schwarz VNA equipment used for measurement and (b) the sensor used for the experimental work.

1 The IDE sensor was fabricated using a industrial standard chemical etching method, with the  
2 patterned electrode being on a Rogers substrate and gold plated to prevent sensor degradation  
3 due to interaction with samples. The design of the IDE allows measurement of the reflected  
4 power from the sensor via the  $S_{11}$  parameter. It accommodates a 400 $\mu$ L fluid sample due to a  
5 well which is bonded to the structure, and the sample is introduced via a pipette.  
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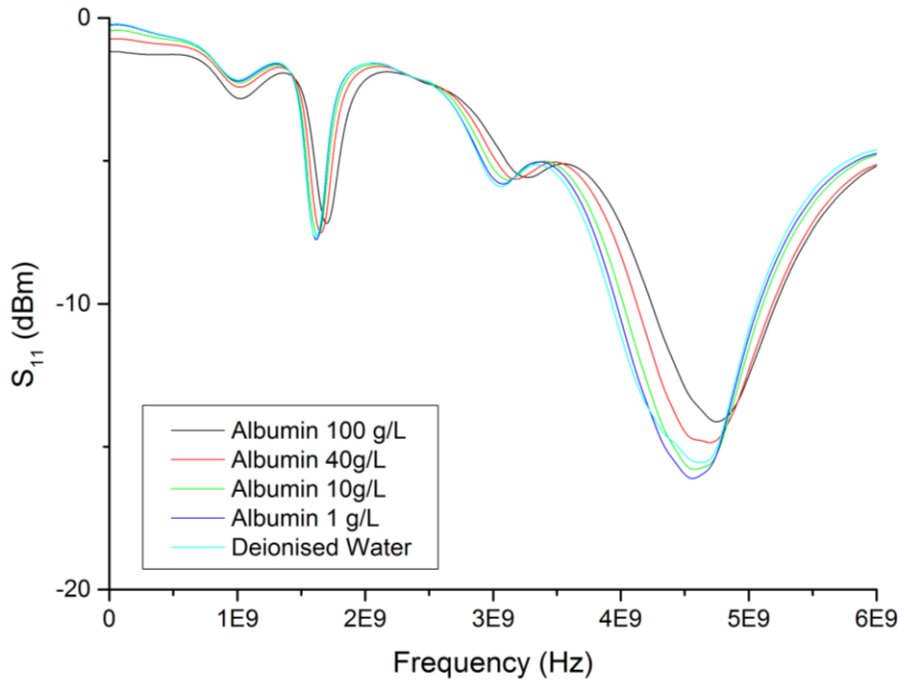
10 The VNA was permitted to scan through all frequencies (10MHz to 15 GHz) at least 3 times  
11 before a recording was made, to allow for sensor output stabilisation. Each measurement  
12 enabled the recording of 60,000 data points in the selected frequency range. Between  
13 samples the IDE was washed with distilled water and left to dry before further measurements  
14 took place.  
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20 Results are analysed and graphs plotted with Origin® 9.1 Data Analysis and Graphing  
21 Software.  
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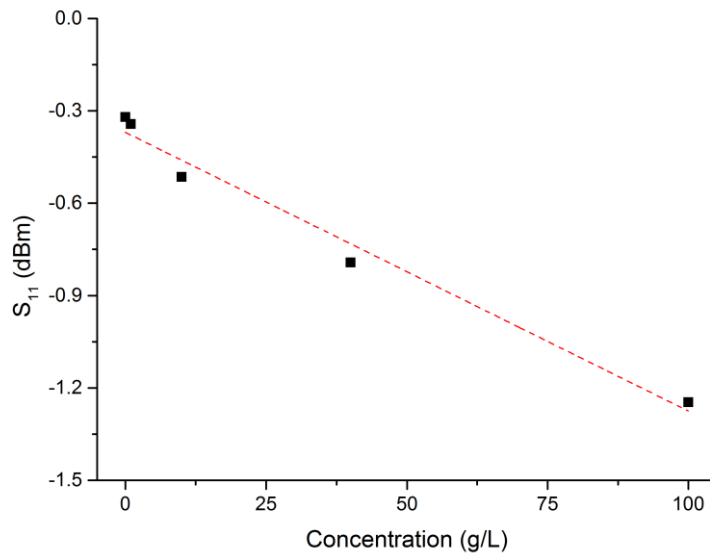
## 28 **Results**

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30 The results obtained have been processed to remove outliers using an average standard  
31 deviation ( $\sigma$ ) method (i.e. where the mean  $\sigma > 2$ , results were discarded). This resulted in  
32 10% of the 50 available measurements being discarded. Figure 2 illustrates the mean spectra  
33 captured during the experimental work, with emphasis being given to the frequency range  
34 between 10 MHz and 6 GHz; beyond these frequencies little change was noted, likely due to  
35 the design of the sensor not favouring higher frequency excitation. This figure shows clear  
36 shifts in the captured signal throughout the displayed spectrum, but this is further highlighted  
37 in Figure 3, namely at 200 MHz and 4 GHz.  
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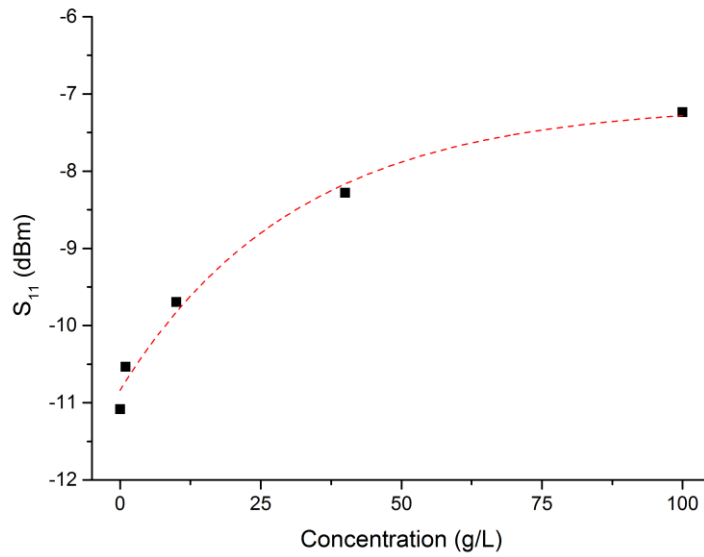




**Figure 2.** Mean spectra captured during experimental work between 10 MHz and 6 GHz.



(a)



(b)

**Figure 3.** Illustrating the relationship between albumin concentration and  $S_{11}$  magnitude (dBm) for the frequencies (a) 200 MHz and (b) 4 GHz.

At 200 MHz, the relationship between albumin concentration and  $S_{11}$  magnitude follows an approximately linear trend, where  $R^2 = 0.991$ . A high degree of repeatability is exhibited, with  $\sigma \leq 0.008$  across all repeated measurements for all dilutions. When comparing the measurements for different dilutions it is noted that  $\sigma = 0.386$ . This demonstrates that while the sensor exhibits very little change between repetitions of the same albumin concentration, as the concentration varies, the sensor output changes significantly.

At 4 GHz an exponential trend is shown. Here,  $R^2 = 0.975$ , repeatability is characterised by  $\sigma \leq 0.259$  across all repeated measurements for all dilutions, and when comparing measurements for different dilutions  $\sigma = 1.591$ .

## Discussion

Although there are many methods used to try and prevent spinal cord ischaemia, the only method of evaluating the integrity of the spinal cord intra-operatively is the use of motor evoked potentials (MEPs). However this technology does not have strong evidence

1 underpinning its use. This is reflected in such that worldwide there is no consensus in its use  
2 and applicability and consequently there is huge variation in how MEP measurements are  
3 used worldwide [14-16].  
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6 There has been a good amount of research regarding potential detectable biomarkers in the  
7 CSF and their correlation to spinal cord ischaemia. Drenger et al measured lactate  
8 concentrations in both CSF and serum during thoracoabdominal aneurysm repair [25].  
9 Patients who became paraplegic showed a greater increase in CSF lactate concentrations after  
10 aortic clamp release compared with those who suffered no neurological damage.  
11 Furthermore, CSF measurement of lactate decreased with strategies used to protect the spinal  
12 cord (hypothermia and distal aortic perfusion). These results have been reproduced in a more  
13 recent study by Casiraghi et al where 4 patients developed SCI, and CSF lactate was  
14 significantly higher before, during and after surgery [32]. Our previous work has already  
15 shown the ability of our sensor to accurately measure lactate at physiological levels with our  
16 sensor with tangible evidence that this could be possible in real time [20-21, 24].  
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27 With time there has been considerable interest of biomarkers that can be biochemically  
28 analysed in CSF that are more sensitive to SCI than lactate. There has been considerable  
29 interest in the S100 $\beta$  protein, which is a glial specific protein expressed primarily by  
30 astrocytes. This protein is secreted in large quantities following the acute phase of brain  
31 trauma and ischaemia. Khaladj et al studied 13 patients undergoing elective  
32 thoracoabdominal aneurysm surgery, and of these 2 developed spinal cord injury [29]. In the  
33 patients that developed spinal cord ischaemia there was a strong correlation between S100 $\beta$   
34 and lactate levels in the CSF compared to those who did not. Anderson et al analysed the CSF  
35 and serum of 11 patients who underwent thoracoabdominal aneurysm surgery and measured  
36 S100 $\beta$ , lactate, neuronal specific endolase, glial fibrillary acidic protein [30]. One patient  
37 suffered stroke following surgery, which cause significant rises in all biochemical markers  
38 measured in the CSF. A further patient who suffer paraplegia again showed significant  
39 increases in all biomarkers. GFPA in this study was found to increase in patients with  
40 neurological damage but not in those without. This finding of increases in GFPA was further  
41 reinforced by a study from the Safi group where GFAP increased before or in parallel to  
42 onset of symptoms in the patients with delayed paraplegia [31]. However, although these  
43 biomarkers show excellent probability for use during TAAA repair they currently require  
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biochemical laboratory analysis which and requires time, expensive equipment and specialist expertise, not conducive to a real time sensor which could be used intra-operatively.

The utilisation of electromagnetic waves, particularly microwaves, has a number of benefits which make this suitable for our current area of research. Firstly, microwaves are non-ionising with a low power output of approximately 1mW (0dBm) making this technology safe for use at the bedside for both patient and operator. Furthermore, the data output can be produced in real time making this an attractive quality during an operation. We are currently able to manufacture these sensors at low cost and believe that this will be reflected in the final product which is important in the current economic climate. The multi-parameter nature of wide band microwave analysis can provide unique spectrum signatures which yields a vast array of data.

We believe that our current results show that microwave analysis can provide a sufficient means of detecting protein over a range of concentrations. Our current experimentation now focuses on calibrating our sensor to detection of different types of proteins in different substrates including human CSF as well as improving the sensitivity and resolution of the sensor. Our current study remains a proof of concept, as although albumin is a protein, it is unlikely in itself to be a marker of spinal cord ischemia. This said however, related work of the authors in agriculture has shown albumin to be a valuable measure of long-term protein level in livestock and thus could well prove open up a new way to determine the effectiveness of diet.

## Conclusions

This work uses an interdigitated electrode (IDE) sensor and shows that it is capable of detecting various concentrations of albumin (from 0 to 100 g/L) with a high degree of repeatability at 200 MHz ( $R^2 = 0.991$ ) and 4 GHz ( $R^2 = 0.975$ ). This work presents a significant step toward the measurement of a broad range of proteins, with the aim of being able to measure them in real-time in order to enhance the success of TAAA repair procedures. This will be a step-change to current measurement procedures which require costly lab-based measurements which are not capable of returning results in a timely fashion. Our future experimentation will focus on improving the sensitivity and resolution of the sensor, as well as testing it on human CSF samples.

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