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A549 Cells Measurements with Optical, Impedance and Microwave Spectroscopy †

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Abstract: Lung cancer, especially lung adenocarcinoma, is one of the main causes of death worldwide. This paper reports on the real-time detection methods of human lung adenocarcinoma A549 cells. The proposed approach is based on using optical, impedance and microwave spectroscopy in an attempt to develop an online tool that can be easily used for early cancer diagnostics and treatment monitoring not only in the hospital labs, but at point of care. Low power athermal electromagnetic waves as a sensing mechanism for early cancer detection showed a particular promise. The techniques were also applied to other cells, such as PC3, LL24, HLF, HACAT and DU145, to ensure selectivity of the sensors. Providing real-time information at point of care will be a significant leap on its own as it will dramatically increase screening capabilities.

Keywords: microwave spectroscopy; cancer cells detection; A549; optical and electrical properties; point of care monitoring

1. Introduction

Lung cancer is one of the most common malignant tumours, and radiotherapy is the prevailing method of choice for treatment, especially for middle- to late-stage lung cancer. Radiation works by damaging the DNA of cancerous cells and altering apoptosis-related genes or proteins, leading to cell death. However, early detection of cancer remains a challenge, as it is normally diagnosed by physical examinations, imaging, and measurement of subtle changes in hormone levels. This is confirmed through invasive tissue biopsy of the tumour, which cannot be performed repeatedly due to the negative impact on patient health and quality of life.

This paper reports on the development of novel technology for early detection of the cells, which cancer uses to spread in the blood (circulating tumour cells: CTCs). If CTCs are detected after cancer has gone into clinical remission, it can be treated immediately. If therapy can eradicate the CTCs before they engraft onto another organ, secondary metastases will be halted, causing cancer survival rates to significantly improve [1]. Cancer cells are known to produce metabolite lactate at higher concentrations than normal cells via a metabolic phenomenon referred to as the Warburg effect. Contemporary research in the field of oncology is heavily focussed on detecting cancers in their earliest possible stages in a minimally invasive format.

EM wave sensors operating at microwave frequencies are seeing an increasing interest for minimally-invasive [2,3] and non-invasive [4–6] medical purposes. The sensors can typically be characterised as requiring low power (<1 mW) while retaining a good level of penetration into a target

material that they may assess properties beneath a surface, even to determine a blood lactate level through the skin of a subject [7].

This paper reports on an alternative screening technology based on detecting small basal changes in a patients resting lactate concentration. The approach is based on the use of low power non-invasive electromagnetic waves, which have already been shown capable of non-invasive transdermal lactate measurement [7]. The optical properties, capacitance measurements and microwave spectroscopy were used to assess the sensitivity of each method.

2. Materials and Methods

For any cancer detection strategy to be of clinical value it needs to be capable of detecting cancerous cells whilst they are a rare cell, giving clinicians the greatest opportunity to intervene before they begin to overcome a tissue. With the goal of elucidating the minimal amount of cells our technology is capable of detecting, the following experiments were constructed: tumour cells were cultured in 4.5 g/L Glucose/+glutamine/+pyruvate with supplementation of 5% Fetal Bovine Serum and 1% Penicillin/Streptomycin (henceforth referred to as growth media) at 37 °C, 5% CO_2 until confluent. When confluent cells were recovered using a 5 min incubation with trypsin (37 °C, 5% CO_2), collected by centrifugation for 5 min at 500G and re-suspended in an appropriate volume of growth media to facilitate counting using a haemocytometer and inverted light microscope.

Appropriate volumes of cell suspension were added to wells of a 24 well plate to yield the following cell quantities: 0, 50, 100, 500, 1000, 5000, 10,000, 20,000, 30,000, 50,000, 75,000, 100,000, 200,000, 300,000, 500,000, 1,000,000 in a final volume of 1 mL growth media.

Cells were cultured for 24 h media was collected, transferred to a clean 24 well plate, and frozen at −80 °C for analysis. Remaining adherent cells were washed using phosphate buffered saline and also frozen at −80 °C for future analysis should they be required. Lactate measurements were also performed spectrophotometrically using a commercially available lactate quantification kit (Sigma-Aldrich, Dorset, UK), following the manufacturer's instructions.

Figures 1 and 2 illustrate the experimental setup for contactless measurement of lactate levels in the solution using EM sensor. Note, the reason for 2 small wells is that one of them can be a reference well, where a calibrating solution can be placed and the response between two wells is contrasted. This is also done for temperature compensation or other uncertainties.

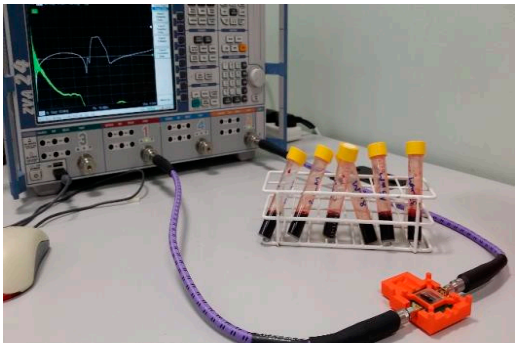

Figure 1. Experimental setup with VNA and 2-port EM sensor connected via coaxial cables.

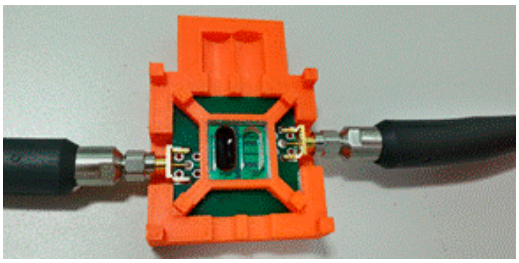

Figure 2. Close look at the developed electromagnetic wave sensor for early cancer detection. Two-port arrangement allows for both S_{11} and S_{21} simultaneous recording.

3. Results and Discussion

The optical properties (Figure 3), capacitance measurements (Figure 4) and microwave spectroscopy (Figure 5) data all showed that each technique is capable of distinguishing various levels of lactate in cell solutions, as illustrated below.

3.1. Optical Properties

Absorbance of the A549 cell solutions was assessed with (Jenway 7315, designed and manufactured by Bibby Scientific Ltd, Stone, Staffs, UK) UV-Vis Spectrophotometer in 300–1000 nm wavelength range. The absorbance spectra gave distinct indication for the highest and lowest lactate levels in whole measured range, the peak region around 560 nm (Figure 3) showed the biggest difference in response between the various solutions. Although this technique is well developed for the laboratory use, it is bulky, time-consuming, and required relatively large volume of solutions to conduct the measurements, which is impractical for point-of-care diagnostics.

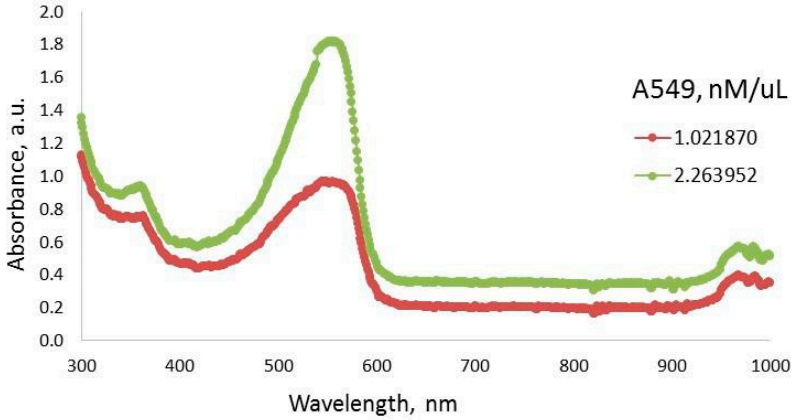

Figure 3. Optical measurement performed with UV-Vis Spectrophotometer: Absorbance of A549 cells solutions change with their concentration.

3.2. Capacitance Measurements

A Hameg 8118 programmable LCR bridge was used to record the change in capacitance of the solutions in 200 Hz–200 kHz frequency range. Figure 4 illustrates linear calibration curves for the capacitance at 200 Hz and 50 kHz with R^2 values being 0.9652 and 0.9864 respectively. This method may be considered the most sensitive to various concentrations of lactate in A549 cells solutions. However, this method required direct contact of the measuring probe with the solution, and therefore is only suitable as a benchmark indicator and not practical for real-life applications.

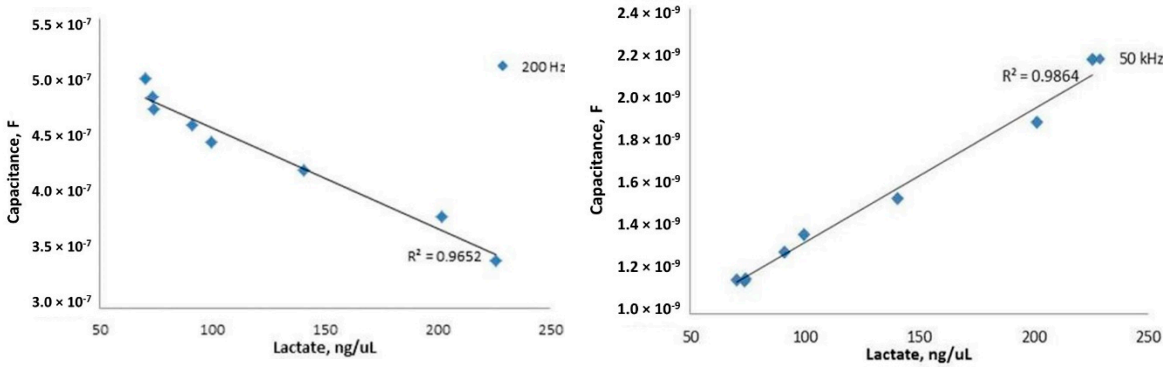

Figure 4. Capacitance calibration curves at 200 Hz (**left**) and 50 kHz (**right**), with R^2 shown.

3.3. Microwave Spectroscopy

In this work, Rohde & Schwarz 24 vector network analyser (VNA) in the frequency range from 10 MHz to 13 GHz was used for contactless lactate measurements as illustrated in Figure 1, namely, the measured solution does not physically touch the sensors, but only the container. Using the full capabilities of the VNA, 60,000 points were recorded for each spectra. Data processing indicated that the reflected power signal S_{21} was the most susceptive to changes as a result of varied lactate level in A549 cells solutions in 1.75 GHz–1.9 GHz range (Figure 5). Notably, further data processing using, for example, neural network method, can reveal other frequencies of interest, which in turn, can be used for system miniaturisation and portable electronics development.

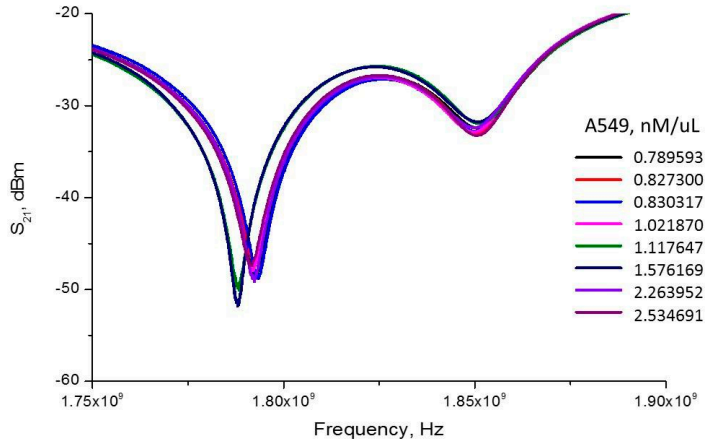

Figure 5. S_{21} measurements for A549 cells samples with known lactate level in 1.75 GHz–1.9 GHz range.

4. Conclusions

It is believed that a bespoke sensing approach based on the use of low power non-invasive electromagnetic waves presented in this paper may provide clinicians with valuable evidence for further, more specific, oncological analysis and ultimately has the potential to become a rate limiting step in stratifying their care.

Author Contributions: A.M. and N.B. conceived the original idea and implemented an appropriate design for the sensor and concept. N.B. has prepared all the cells cultures, O.K. has conducted the measurements. Together with A.M., O.K. has processed the results and written the paper.

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Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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