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Lipid droplet detection by the cavity perturbation method

R T Blakey[1], A Mason[1], C E Rolph[2], G Bond[3] and A I Al-Shamma'a[1]

[1] School of Built Environment, Liverpool John Moores University, Liverpool L3 3AF, UK
[2] School of Pharmacy and Biomedical Sciences, University of Central Lancashire, Preston PR1 2HE, UK
[3] School of Forensic and Investigative Sciences, University of Central Lancashire, Preston PR1 2HE, UK

Email: r.t.blakey@2010.ljmu.ac.uk

Abstract. There are currently no point-of-care diagnosis strategies available to indicate the presence of neoplasmic growth. This research aims to develop a novel diagnostic strategy based on detecting TAG accumulation in cells. This element of the research is a preliminary experiment to prove the concept of detecting TAG lipid droplets in YEPD media. It was found that a change in mono-unsaturated concentration can be detected by the frequency shift in a resonant cavity. The dielectric constant of TAG vegetable oils was calculated at 2.34-2.39. It was also found that concentrations of lipid droplet can be differentiated up to 5% (v/v).

1. Introduction

Despite decades of research, cancers or malignant neoplasms continue to significantly contribute to patient morbidity and mortality. Neoplasms occur when cell proliferation exceeds cell apoptosis (naturally initiated cell death) which results in the formation of a tumour or increased blood cell volume depending on the origin of the cancerous cell. Tumours can be problematic to detect in the early stages due to symptoms not arising until later stages of development. When tumours are detected in earlier stages treatments become less invasive, simpler and cheaper resulting in increased patient comfort and prognosis. Screening programs have been extremely successful in lowering the incidence of late stage cancers (e.g. breast [1], cervical [2]) but are limited to a specific type and site of cancer also requiring specialised facilities, staff and transport (i.e. laboratories). Currently there are no quantitative or qualitative point-of-care diagnostic assays available to indicate neoplasmic tissue. This communication proposes a novel diagnostic strategy that can detect a tumour in the body from any origin.

This is proposed through the measurement of triacylglycerol (TAG) stores within cancerous cells and specialised cells of the immune system called dendritic cells. Under normal circumstances, most cells store small amounts of TAGs in organelles called lipid droplets (lipid bodies, adiposomes) and are used to synthesise lipid membranes, other molecules of the lipid family and as a source of

[1] r.t.blakey@2010.ljmu.ac.uk

chemical energy. However, due to cellular mutations and/or abnormal cell to cell signalling, the metabolism of the cell is altered resulting in TAGs being accumulated in such lipid droplets. Depending on the mutations that lead to the neoplasm, a lipid droplet can constitute a significant sum of cell volume [3]. Hence, the cell concentration of TAG is an excellent indicator or biomarker of cell metabolism and proliferative state. Furthermore, excess TAGs released into circulation are "mopped up" by cells of the immune system. Dendritic cells are specialised cells of the immune system which scavenge for non-self or otherwise foreign molecules and present digested material to other cells of the immune system (T-cells). Recent research has found that dendritic cells from tumour bearers accumulate TAG due to exacerbated macrophage scavenger receptor 1(MSR1) mediated uptake [4]. This relationship is complex and it is unclear whether TAG accumulation inhibits the immunogenic actions of dendritic cells facilitating the growth of tumours or the result of a tumour being present. Dendritic cells have the advantage of being present in the blood stream while migrating to specific sites around the body allowing cells to be easily isolated from the body (e.g. blood sample) to be characterised, removing the need for invasive diagnostic strategies such as surgery or biopsy. Lipid droplet accumulation also occurs in a range of cells specifically related to conditions of infection and inflammation [3].

Many different impedance or dielectric spectroscopy techniques have been developed in an array of cancer diagnostic applications and show promising results. Approaches vary depending on the site and type of cancer from characterising isolated individual cells to non-invasive *in-vivo* imaging techniques [5-7]. Dielectric spectroscopy analyses of cellular material are based on dielectric properties at different frequencies. Biological cells exhibit a characteristic decrease in permittivity as a function of increasing frequency known as α, β, δ and γ dispersions [8]. The aforementioned review is particularly useful to the biologist and engineer alike who may not be familiar with electromagnetic biological cell interactions.

The following experiment provides a simple preliminary proof of concept trial to determine if different conformations of TAGs and different concentrations of TAG droplets can be differentiated based on their dielectric properties at microwave frequencies. This is calculated using the small perturbation method whereby a sample introduced to a resonating cavity shifts the frequency of resonant modes and decreases the quality factor dependant on the dielectric characteristics of the sample under test [9-11]. Resonant based sensors have proved to be extremely efficient in detecting the small changes in the biochemical make up and biological characteristics of a cell which can be calculated based on the measured electromagnetic properties of the cell sample [12].

2. Methods

2.1. Chemicals and reagents

Yeast extract, bacto-peptone, dextrose (YEPD) were purchased from Sigma-Aldrich UK. Grapeseed, Groundnut, Olive and Sunflower Oils were purchased as non-branded products from a well known U.K. supermarket.

2.2. Preparation of TAG and TAG lipid droplet samples

Samples of 100% grapeseed, groundnut, olive and sunflower oil were prepared in sample tubes ready for dielectric measurement. Secondly, olive oil was suspended in YEPD media to yield concentrations of 50, 10, 5, 1, and 0.5 (% v/v) oil. YEPD was prepared to the following protocol: 1 % (w/v) of yeast extract, 2 % (w/v) of bacto-peptone and 2 % (w/v) of dextrose diluted in distilled water. Before measurement, the tube to be sampled would be vortex until a homologous suspension was achieved.

2.3. Dielectric measurement and calculation

Measurements were performed using an Agilent Technologies (Hewlett Packard) 8720 ET Vector Network Analyser (VNA). The instrument was set to generate a signal between 1.5-1.9 GHz over 1601 data points and calculate the S_{21} (transmission) parameters. Measurements were made through a

custom cylindrical resonating cavity, designed and constructed in house. The cavity was designed to resonate at 1.7 GHz at mode TM_{010}.

The dielectric constant, dielectric loss and loss tangent of the sample were then calculated using the following equations [11].

$$\varepsilon' = \frac{V_c\,(f_c - f_s)}{2V_s\,f_s} + 1 \tag{1}$$

$$\varepsilon'' = \frac{V_c}{4V_s}\left(\frac{1}{Q_s} - \frac{1}{Q_c}\right) \tag{2}$$

$$\tan\delta = \frac{\varepsilon''}{\varepsilon'} \tag{3}$$

where ε' and ε'' is the dielectric constant and dielectric loss respectively, f_c and f_s are the resonant frequencies of the empty cavity and loaded cavity respectively, V_s and V_c are the volumes of the sample and cavity respectively and Q_c and Q_s are the quality factors of the empty cavity and the loaded cavity respectively.

3. Results

Figure 1 shows the S_{21} parameters for the vegetable oil standards and empty cavity as a function of frequency illustrating frequency perturbation for groundnut oil of 17.1 MHz, olive oil of 17.4 MHz, sunflower oil of 17.5 MHz and grapeseed oil by 17.7 MHz to 1.69 GHz.

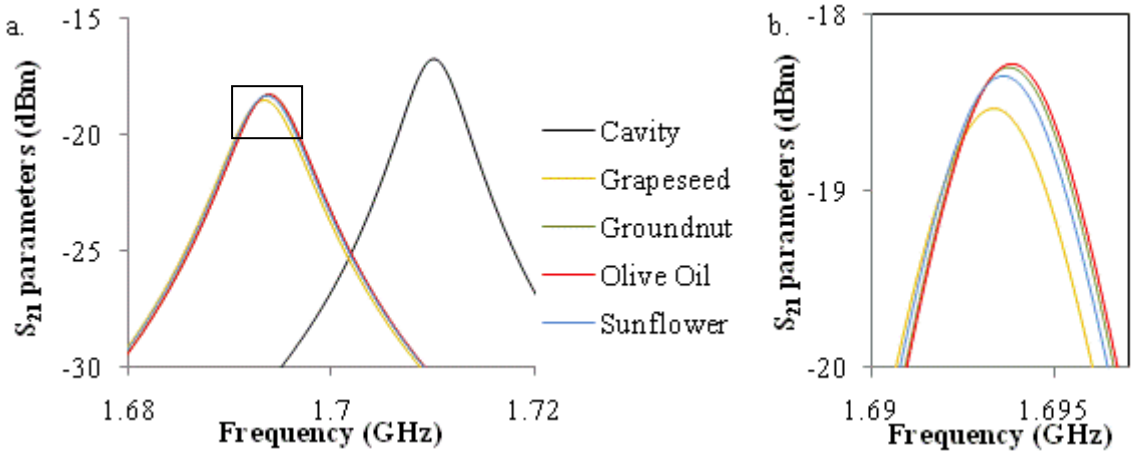

Figure 1a. S_{21} parameters of TAG samples (magnified in figure 1b.) and the unloaded cavity. S_{21} parameter frequency spans from 1.68 to 1.72 GHz.

Figure 2 shows the S_{21} parameters of differing concentrations of Olive Oil suspended in YEPD. It can be seen that as the concentration of olive oil decreases, the resonant frequency of the sample decreases or shifts to the left away from the resonant frequency of the empty cavity. Results can be seen in table 1. The total frequency shift between the empty cavity and 0.5% oil is 135 MHz to 1.576 GHz (Peak E). Peak D and E consist of two curves on the below graph, 10% -5% and 1%-0.5% respectively. The quality factor of the detected parameters decreases as YEPD media is introduced into the sample.

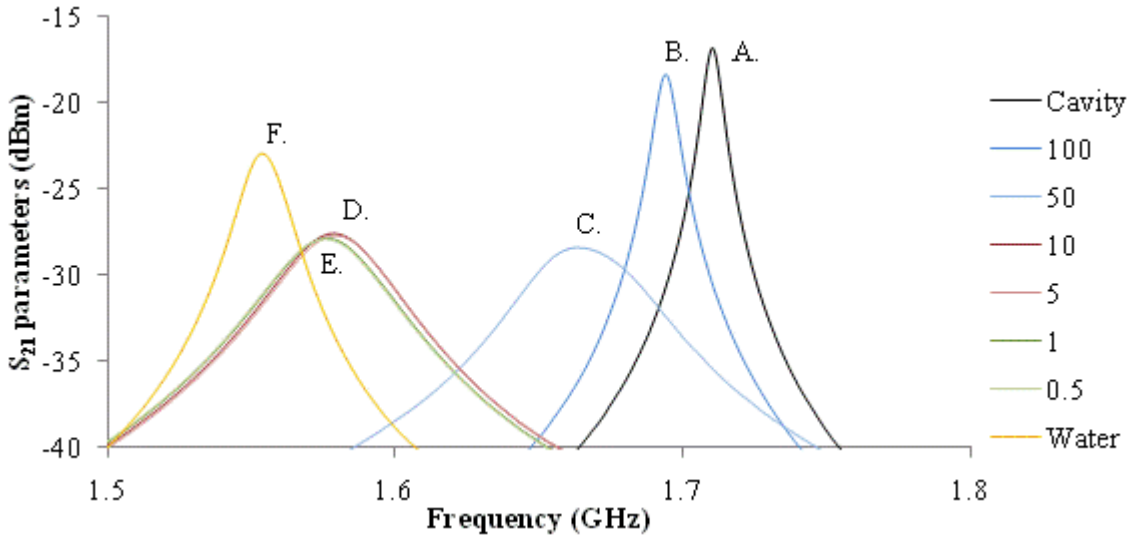

Figure 2. S_{21} parameters of lipid droplets present in differing concentrations (olive oil % v/v). Peaks from right to left are A: Cavity. B: 100%. C: 50%. D: 10% and 5%. E: 1% and 0.5%. F: Water.

3.1 Dielectric calculations

The dielectric constant, dielectric loss and loss tangent of the samples were calculated using (1-3). The same parameters were also calculated for the TAG-YEPD suspensions. The results can be seen below in table 1. It can be seen that grapeseed and sunflower oil have the greatest shift in frequency resulting in a higher dielectric constant. This is due to the levels of unsaturated acyl chains present on TAG molecules that make up the oil. Groundnut and Olive Oil are composed of approximately 53% (w/w) and 72% (w/w) monounsaturated fatty acids respectively as compared to 21% (w/w) and 16% (w/w) of sunflower and grapeseed oil. This changes the dielectric characteristics of the sample resulting in a frequency shift. This is in agreement with previous research that states the dielectric constant of TAGs increases as the level of unsaturated fatty acids (FA) increases [13]. The Q-factor of the oil samples decreases indicating an increase in dielectric loss.

Table 1. Calculated dielectric properties of oil samples and suspensions.

	Frequency shift (MHz)	**Q-factor**	**Dielectric constant**	**Dielectric loss**	**Loss tangent**
TAG					
Grapeseed	17.7	199.21	2.387	0.100	0.003
Groundnut	17.1	199.28	2.340	0.100	0.004
Olive	17.4	199.25	2.364	0.100	0.004
Sunflower	17.5	201.61	2.372	0.006	0.004
Olive Oil (% v/v)					
100	17.0	199.25	2.364	0.100	0.003
50	47.0	35.03	4.749	1.572	0.331
10	132.5	38.04	12.142	1.422	0.117
5	132.0	38.99	12.096	1.380	0.114
1	134.5	38.93	12.324	1.382	0.112
0.5	135.0	39.40	12.370	1.362	0.110

The dielectric constant of the oil suspensions increases as the concentration of YEPD increases. This is due to the water content of the media shifting the frequency closer to that of the water standard. Dielectric losses also increase as YEPD is introduced. This is due to the ion content of the media resulting in energy being lost as dielectric heating.

4. Conclusions and Discussion

This experiment has proved that differing conformations of lipids and different concentrations of lipid droplets can be distinguished based on the electromagnetic properties of the suspensions which are apparent in the frequency shift of the samples at this frequency range. However, limitations include the sample size being too large for this method. Accuracy of the technique increases as the sample size decreases. The technique also has the drawback of being inaccurate on low loss samples [11]. The current resonator and sample volumes are sensitive enough to detect lipid droplet concentration up to 5% (v/v). Further modification of the resonator cavity used to detect the lipid droplets will positively advance the accuracy of the calculations involved with this method. The homogeneity of the sample may also have introduced errors. As the sample is introduced to the cavity the TAG droplets begin to converge into larger droplets until the TAG begins to separate into a different phase resting on the YEPD media. This results in the lipid droplet concentration being measured actually being lower than the newly vortex suspension. Stabilisation of the lipid droplets using phospholipids would improve the homogeneity of the suspensions improving measurement accuracy.

The next stage of the research will look to detect lipid droplets within living yeast cell cultures. A number of complex theoretical models exist that can calculate the size and shape of the cell and composition of the cytoplasm including organelles from the dielectric characteristics of a sample. Such models will prove to be crucial in the correct determination of TAG accumulation within living cells.

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