

MICROWAVE DIELECTRIC SPECTROSCOPY OF LOW-VOLUME FRACTION HUMAN CANCER CELLS EMBEDDED IN COLLAGEN GELS

Experimental Feasibility Study with an Open-ended Coaxial Probe

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Abstract: This paper addresses and demonstrates the feasibility for microwave dielectric spectroscopy to detect small volume fractions of SK-MES lung cancer cells embedded in collagen gels with an open-ended coaxial probe. Measurements were performed on the frequency range 200 MHz – 2 GHz. For all the cell volume fractions tested (1.4%-4.4%), a significant difference in complex permittivity was observed between composite gels (containing cells) compared to gels alone. Statistically significant changes were especially found in the real part of the permittivity, which decreased consistently when the volume fraction increased.

1 INTRODUCTION

The dielectric properties of biological tissues and cells have been widely investigated for many decades (Foster, 1996; Gabriel 1996). They are characterized by the so-called complex permittivity, expressing the polarization response of a material in the presence of a time-varying applied electric field. Measuring its complex response as a function of the field frequency is referred to as dielectric spectroscopy (DS).

The difference in the dielectric properties between various biological tissues is exploited in electromagnetic tomography techniques, such as biomedical microwave imaging, a promising imaging modality (Tofighi, 2001; Semenov, 2003; Fear, 2005). One of its particular interests is the ability to detect tumours (Hagness, 1998; Bulyshev, 2001; Shao, 2005; Bindu, 2006). Indeed malignant tissues have mainly been found to have significantly different dielectric properties than the corresponding normal tissues regarding both the real and the imaginary parts of the complex permittivity (Chaudary, 1984; Surowiec, 1988; Smith, 1986; Joines, 1994; Sha, 2002). In this regard, DS studies

related to cancer have mostly dealt with bulk tissues. In these kinds of studies bulk tissues are either investigated *in vitro* or *in vivo* which have both disadvantages. *In vitro* studies have mostly investigated non-living tissues. Moreover it is difficult to deal with *in vivo* human tissues mainly because surgery is needed, and it does not necessarily give information at cell level.

On the contrary, working on cell culture samples is a good alternative way to get a better biophysical knowledge of living cells. In this regard, a number of DS experiments have been carried out on various types of cell suspensions. The most commonly investigated cell suspensions, whatever the volume fraction (ranging from a few percent to roughly 70%), have logically been blood samples (Lisin, 1996; Chelidze, 2002; Bordi 2002; Jaspard, 2003; Treo, 2005) and yeast suspensions (Claycomb, 2002). A few of them have especially focused on measuring the dielectric properties of white blood cancer cells in suspension which were shown to be also different from those of normal cells (Polevaya, 1999; Ermolina, 2001). Furthermore, *in vitro* cell culture samples embedded in microporous scaffolds have also been successfully investigated for tissue engineering purposes (Bagnaninchi, 2003 and 2004).

The method was proved to be a good way to monitor cell growth and differentiation in scaffolds used in tissue engineering. The aforementioned studies dealing with cell culture samples were shown to be able to retrieve cell signature. This term refers to the dielectric properties of the main cell compartments, such as membrane, cytoplasm but also nucleus. These so-called cell signatures were shown to be distinct for different cell lines or types. This property allows for cell separation of cancer cells from normal cells by dielectrophoresis (Gascoygne, 1997). In these different studies, measurements have covered different parts of the frequency spectrum, extending from the extremely low frequencies to the lower part of the microwave range depending on the measurement method used.

The motivations of the present study are mainly threefold. The first aim would be to get a better biophysical understanding of the differences in dielectric properties between epithelial lung cancer cells and the corresponding normal ones. Secondly, DS could also be used as a method to analyse the response and effectiveness of various anti-cancer treatments such as chemotherapy drugs on *in vitro* cell culture samples. Some authors have already used DS in the radio and microwave frequency range to do so (Santini, 1991 and 1995; Hübner 2005; Duncan, 2006). Thirdly, this study is a preliminary step to investigate the capability of DS to be used on intraoperative tissue biopsies for on-line assessment of tissue resection effectiveness.

To address these issues, our approach is an adjunct approach of cell suspensions and allows to have an *in vitro* realistic biological 3D model for living lung epithelial cells. As a relatively dense matrix, a collagen gel is a good model for investigation. Collagen is the major component of the natural extracellular matrix (Pietrucha, 2005) and has already been used in other biomedical studies involving similar lung cancer cell culture samples (Yang, 2004). We used low volume fractions of cells in order to have an *in vitro* system that could detect small number of cells so this could have the clinical application of detecting tumours when they are still small enough to undergo radical treatment.

This paper deals with the preliminary investigation of the dielectric properties of lung cancer cell samples embedded in collagen gels in the lower part of the microwave range (UHF). The cells in question belong to a human epithelial lung cancer cell line, namely SK-MES cell line. This paper especially addresses the feasibility of distinguishing low-volume fraction of these cells from the matrix in

which they are embedded in this part of the frequency spectrum.

The first section describes in details the materials and experimental set-up used and the second section gives and discusses some obtained results.

2 MATERIALS AND METHODS

We mainly conducted 6 different experiments, on 6 different cell volume fractions: 1.4%, 1.9%, 2.7%, 3.2%, 3.8%, 4.4% corresponding respectively to about 4, 5.3, 7.4, 9, 10.6, 12.3 million cells per gel. They were counted with the aid of a grid-counting chamber (Hycor Kova Glasstic). The volume fraction was estimated by supposing the cells are spherical. Their diameter (mean: 18 μm \pm standard deviation: 2.2 μm) was measured on a slide with a light microscope with a computerized ruler.

For each of the 6 experiments, two sets of 7 collagen gels were prepared and measured. The first set did not include any cells and the second set included the same volume fraction of SK-MES cells for a given experiment.

2.1 SK-MES Cell Culture

SK-MES cells (ECACC, UK) were cultured in 175 cm^2 -cell culture flasks and incubated at 37°C and 5% CO_2 . Each culture vessel contained complete culture medium composed of high-glucose Dulbecco's Modified Eagle's Medium supplemented by 10% volume of foetal calf serum and other standard components according to the provider's instructions and previous studies (Yang, 2004).

2.2 Collagen Gels Preparation

Collagen type I gels were prepared according to the supplier's (BD Biosciences) instructions. To ensure the viability of SK-MES cells reported in (Yang, 2004), the concentration of collagen was 1.5 mg/mL.

Cells were added and mixed with the gel at a temperature of 4°C when collagen is in liquid form. Gels were allowed to set by incubation at 37°C for 3 hours.

Each gel was prepared in a cylinder well and had a diameter of 19 mm and a height of 3 mm. These dimensions are justified in the next section. Therefore each gel had a volume of 0.85 mL.

2.3 Experimental Set-up

2.3.1 Experimental Material

Dielectric spectroscopy was performed using a vector network analyser (model 8753E, Agilent Technologies) operated on the frequency range 200 MHz – 2 GHz connected to a flanged open-ended coaxial probe (dielectric probe, Agilent model 85070) via a coaxial cable.

The complex permittivity of the sample is actually deduced by calculation from the measurement of the complex reflection coefficient at the tip of the probe. Indeed the reflection coefficient is linked to the impedance or admittance seen at the tip of the probe, which is itself directly related to the complex permittivity of the sample by a suitable model (implemented by Agilent's software).

In theory, the model supposes that the sample is semi-infinite (i.e. covers a half space) isotropic and homogeneous. In practice, if the sample is not homogeneous (our case), the result is an average value weighted by the pattern of intensity of the electric field (which is highest at the centre of the probe tip). Besides, the sample is always of finite size. In the probe supplier's data sheet, the diameter of the sample must be at least that of the probe, and its minimal thickness is given by a simple formula related to the permittivity, which in our case yields about 2.5 mm. This is in good agreement with values found in scientific papers to measure biological tissues with probes of similar dimensions (Semenov, 2000; Hagl, 2003). Indeed, some researchers have shown that measurement errors are small when the sample thickness is at least as big as the outer conductor radius of the probe (Fan, 1990; De Langhe, 1994; Hoshina 2001), which is 1.5mm in our case. We therefore decided to prepare 3mm-thick collagen gels.

Nevertheless, a small sample size can be problematic in the microwave range especially when its permittivity is high and its losses relatively low, because cavity resonances could occur. This phenomenon explained by electromagnetic cavity theory has been pointed out by some investigators using the same kind of probe (Grant, 1989; De Langhe, 1994; Sheen, 1999). To avoid potential resonance effects, we chose 2 GHz as the upper frequency (until which we did not observe any resonance effect).

Besides, the frequency range of the probe using a network analyser and the supplier's software is guaranteed from 200 MHz to 20 GHz. As a result, we chose the frequency range 200 MHz – 2 GHz.

As shown in Figure 1, gel samples were actually put onto the probe and fitted its dimension, as the outer diameter of the probe flange is 19mm. Several attempts were made to measure the gels from the top with the probe upside down (compared to Figure 1), but the repeatability of the measurement was very poor. Moreover, the gels measured from the top got squashed. On the contrary, putting the gel onto the probe keeps its integrity and allows for a good control of the contact between the gel and the probe, and improves the repeatability of the measurement.

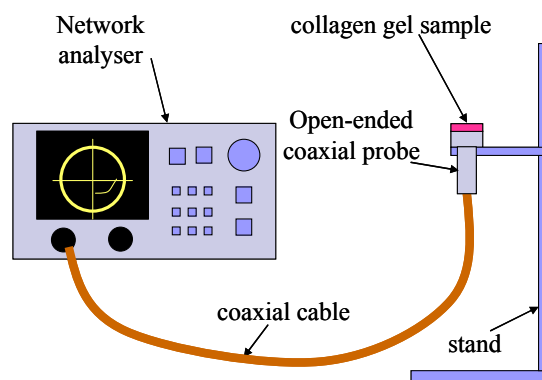


Figure 1: Diagram of the experimental set-up.

2.3.2 Experimental Method

For each experiment, 7 gels with cells, and 7 gels without cells ('controls') were made and measured. Each gel was measured 7 times in order to address the repeatability issue. Prior to measurement, the system was calibrated using a standard procedure, in which the standards are air, a short circuit and deionised water. The latter was measured in a 250 mL beaker to avoid resonance phenomena, in accordance with (Blackham, 1997).

The measurement method was as follows. The wells containing the gels were taken out of the incubator and placed in a water bath at 37°C. Each gel was then put onto the probe, which was at room temperature. To achieve as good repeatability as possible, a few minutes were necessary to get very stable results. On the one hand, while the gels cool down towards room temperature, we observed that the real part of the permittivity ϵ' increased slightly, and the imaginary part ϵ'' decreased slightly on the whole chosen frequency range. On the other hand, when left several tens of minutes at room temperature, they start to dry out and we observed that ϵ' started to decrease and ϵ'' started to increase on the whole chosen frequency range. Measurements were taken at the time when the two aforementioned

phenomena compensate each other. Thus, excellent repeatability was achieved: at the very most, the fractional error (standard deviation divided by the mean of 7 measurements on the same gel) was 0.25% for ϵ' and 1% ϵ'' . If measurements are taken before stabilization, these figures become respectively 0.6% and 2.5% at the very most.

3 RESULTS AND DISCUSSION

The measurement reproducibility was assessed for each volume fraction tested by calculating the fractional error (standard deviation divided by the mean, both calculated on 7 gels measured 7 times, i.e. on 49 measurements) as a function of frequency. At the very most, it reached 0.9% for ϵ' and 5% for ϵ'' . The latter was higher in the lower part of the explored frequency range, where the conductivity is particularly high. However, it could reach 3.3% around 2 GHz.

For all the cell volume fractions tested, a significant difference in permittivity was observed between composite gels (containing cells) compared to gels alone. An example of result for a volume fraction of 4.4% is given on Figure 2 in the complex plane (Cole-Cole diagram).

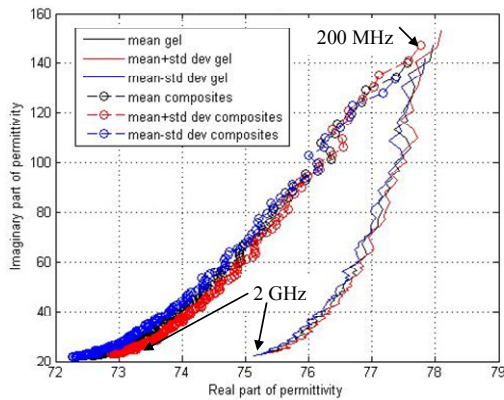


Figure 2: Comparison composite gels / pure gels in the complex plane (mean +/- standard deviation: data obtained on 7 gel samples of each type); volume fraction: 4.4%.

The real part ϵ' was found to be lower when the gels contained cells rather than when they did not (cf. Figure 3), and also to consistently decrease with the cell volume fraction. A statistically significant difference between composites and pure gels was found for all volume fractions. This was demonstrated by a two-tailed Student's t-test (p-value lower than 0.05 or even 0.01 on the major part

of the frequency range). Moreover, the difference in real part between two adjacent volume fractions was also found to be statistically significant by a two-tailed t-test despite the close proximity of the observed variations. It is in good agreement with (Bagnaninchi, 2004) stating that a variation of 0.5% volume fraction is detectable for low cell volume fractions. The p-values were even lower for differences in volume fractions greater than 1%.

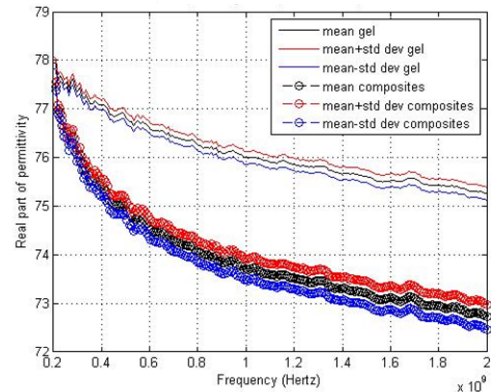


Figure 3: Comparison composite gels / pure gels. Real part of permittivity (mean +/- standard deviation: data obtained on 7 gel samples of each type); volume fraction: 4.4%.

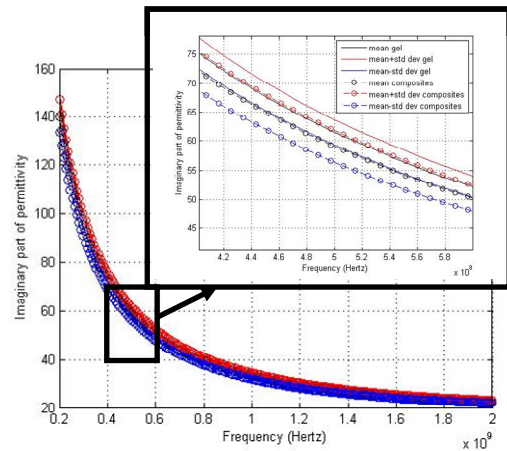


Figure 4: Comparison composite gels / pure gels for the imaginary part of permittivity (mean +/- standard deviation: data obtained on 7 gel samples of each type); volume fraction: 4.4%.

Regarding the imaginary part of the permittivity, a group of 3 experiments (out of 6) showed an increase in ϵ'' when comparing composites and pure gels, and the 3 others showed a decrease (when considering the means). A two-tailed t-test proved that 2 variations among them were not statistically

significant (one in each group; $p > 0.1$ and 0.3), an example of which is shown on Figure 4.

This result about the imaginary part ϵ'' can be commented on qualitatively as follows. This suggests that ϵ'' of the composite gels could actually be of the same order of magnitude as ϵ'' of pure gels because the reproducibility fractional error on ϵ'' (which could reach 5% in the lower frequencies) is not negligible. As gels are quite conductive, ϵ'' is a sensitive parameter whose variability is not negligible.

Quantitatively we did some modelling to explain why ϵ'' of the composite gels can either be a bit lower or higher than ϵ'' of pure gels. We used effective medium approximations commonly utilised with biological cells, such as Maxwell-Wagner or Looyenga equations (Bordi, 2002; Asami, 2002). A composite gel is considered as an effective medium in which cells are inclusions. A basic single-shell cell model, modelling the membrane and cytoplasm by their respective permittivities and conductivities was implemented. The latter were varied even beyond their commonly accepted values: the permittivity of the membrane and the cytoplasm were respectively varied from 2 to 30 and from 30 to 70. The simulation easily shows that for small volume fractions, the conductivity of the cytoplasm is decisive: if it is lower (respectively greater) than the one of pure gel, ϵ'' is lower (respectively greater) for composite than for pure gel. Thus, owing to the variability of the cytoplasm properties, ϵ'' of composite gels can be a bit lower or higher than ϵ'' of pure gels. Hence, the conductivity of the cytoplasm of the measured SK-MES cells could be of the same order of magnitude as that of the measured gels.

Another study (Bagnaninchi, 2003) carried out on the same frequency range but with macrophages put inside chitosan scaffolds filled with a similar ionic culture medium (RPMI) showed different results. The addition of cells induced an increase in ϵ' and a decrease in ϵ'' . However, the effective dielectric behaviour depends on the particular dielectric properties of each type of cells and of the surrounding media. The former constitute the next step of the study.

4 CONCLUSIONS - PROSPECTS

This study has proven the feasibility of detecting small volume fractions of lung cancer cells embedded in collagen gels by microwave dielectric

spectroscopy. The real part of the permittivity was found to decrease with the presence of cells. The imaginary part did not significantly show a consistent variation.

The prospects of this study are mainly threefold. Firstly, further suitable modelling should be developed to try to retrieve cell signature and properties. Secondly, similar experiments should be carried out with the corresponding normal lung epithelial cells and results compared to this study. Thirdly, other DS experiments should also be tried to analyse the response and effectiveness of various anti-cancer treatments such as chemotherapy drugs on *in vitro* cell culture samples.

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