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Dielectric spectroscopy of normal and malignant human lung cells at ultra-high frequencies

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Abstract

Microwave techniques for biomedical applications aimed at cancer treatment or diagnosis, either by imaging or spectroscopy, are promising. Their use relies on knowledge of the dielectric properties of tissues, especially on a detectable difference between malignant and normal tissues. As most studies investigated the dielectric properties of *ex vivo* tissues, there is a need for better biophysical understanding of human tissues in their living state. As an essential component of tissues, cells represent valuable objects of analysis. The approach developed in this study is an investigation at cell level. Its aim was to compare human lung normal and malignant cells by dielectric spectroscopy in the beginning of the microwave range, where such information is of substantial biomedical importance. These cells were embedded in small and low-conductivity agarose hydrogels and laid on an open-ended coaxial probe connected to a vector network analyser operated from 200 MHz to 2 GHz. The comparison between normal and malignant cells was drawn using the variation of measured dielectric properties and fitting the measurements using the Maxwell–Wagner equation. Both methods revealed slight differences between the two cell lines, which were statistically significant regarding conductivities of composite gels and cells.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Recently developed biomedical techniques using microwaves for cancer detection and treatment mainly operate in ultra-high frequencies (UHF), ranging from 300 MHz to 3 GHz. Biomedical microwave imaging, a promising modality for cancer diagnosis, has typically

experimented with frequencies from 800 MHz to 3 GHz (Meaney *et al* 2007, Semenov *et al* 2003, Fear 2005, Li *et al* 2005, Hagness *et al* 1998, Bulyshev *et al* 2001, Bindu *et al* 2006); microwave hyperthermia has been investigated for tumour ablation, mainly using ISM frequencies such as 915 MHz or 2.45 GHz (Converse *et al* 2004, Sherar *et al* 2004, Bertram *et al* 2006).

The development of these two techniques partly depends on the accurate knowledge of the dielectric properties of tissues on which these modalities lie. In this respect, malignant tissues have mainly been shown to have significantly different dielectric properties than the corresponding normal tissues regarding both the real and the imaginary parts of the complex permittivity (Chaudhary *et al* 1984, Surowiec *et al* 1988, Smith *et al* 1986, Joines *et al* 1994, Sha *et al* 2002, Mehta *et al* 2006). These differences were notably found to be significant for fatty breast tissue, for which both permittivity and conductivity were found to be higher in malignant tissues than in normal tissues. Nevertheless, most of these studies were carried out *ex vivo* for convenience or because *in vivo* experiments are not straightforward to perform, especially on humans. Yet dielectric properties measured *ex vivo* do not necessarily reflect the ones *in vivo*. Very recent microwave dielectric spectroscopy studies have completed dielectric data obtained on excised tissues (Lazebnik *et al* 2006, 2007a); some confirmed the aforementioned trends regarding normal versus malignant tissues (Lazebnik *et al* 2007b). However, O'Rourke *et al* (2007) showed that, although *ex vivo* malignant liver tissues exhibited higher dielectric properties than normal tissues, no statistically significant differences were observed in their *in vivo* counterparts. Moreover, the wideband trends of *in vivo* and *ex vivo* dielectric spectra were found to be very different. The authors conclude that 'further work must be carried out to uncover any possible biophysical mechanisms responsible for these differences'. Thus, there is a need for better biophysical knowledge and understanding of the dielectric properties of tissues in the UHF range, especially in their living state.

In this regard, despite its complex structure, a living tissue can be considered to be composed of cells and an extracellular matrix, to which we can add the blood supply. In a rather packed configuration the cells occupy most of the volume fraction (60–70%) of the tissue. The latter parameter is important in determining the dielectric behaviour of a mixture—here, the tissue. Therefore, to gain an insight into the dielectric properties of living tissue, it appears fundamental to know those of its cells. This allows us to assess the contribution of the cells towards the dielectric properties of the tissue, relatively to the role of both the extracellular matrix and the blood supply. A comparison of the dielectric behaviour of normal cells with cancer cells in the UHF range would tell whether or not the cells behave the same way as the corresponding normal versus cancer tissue in this very frequency range. In the latter case, it would mean that the extracellular matrix and the blood supply play a major role in the dielectric properties of the tissue.

Dielectric spectroscopy studies related to living cells have been a good way to obtain better biophysical knowledge at cell level. A number of experiments have been carried out on various types of cell suspensions, for instance on red blood cells (Lisin *et al* 1996, Bonincontro *et al* 1983, Bordi *et al* 2002) or white blood cells (Bordi *et al* 1993, Irimajiri *et al* 1978, 1987). These studies have shown that different types of cells have different dielectric behaviours and properties (Asami *et al* 1989). From the dielectric dispersion(s) present on the spectra, suitable modelling of a cell can extract the permittivity and conductivity of its compartments—plasma membrane, cytoplasm, nuclear envelope and nucleus when appropriate (Irimajiri *et al* 1979, Ermolina *et al* 2000). Because these dispersions occur below the UHF range, measurements have mainly covered lower parts of the frequency spectrum, extending from the extremely low to radio frequencies. To date a major dielectric study (Polevaya *et al* 1999, Ermolina *et al* 2001) has been carried out to compare malignant white blood cells with their normal

counterparts. The results showed significantly different dielectric properties between normal and malignant white blood cells. This is developed further in section 5.

Only a few studies dealing with cell suspensions have focused on the microwave range or ranges straddling radio frequencies and microwaves. Yet, Bonincontro *et al* (1983) were restricted to a single frequency (10 GHz) and Bao *et al* (1994) considered the properties of erythrocytes suspensions but did not focus on the cells themselves. Nonetheless, Bagnaninchi *et al* (2003, 2004) examined a method using an open-ended coaxial probe to measure the complex permittivity of *in vitro* cell culture samples in microporous scaffolds for tissue engineering purposes. For different cell lines, they reported different values of the dielectric properties of cell compartments, giving an indication that this method could potentially distinguish different cell lines embedded in scaffolds in the radio/UHF frequency range.

In the light of latter work, the present study focuses on comparing the dielectric behaviour of normal and cancer cells in the UHF range by using the open-ended coaxial probe technique. Instead of dealing with liquid cell suspensions, the cells were embedded in low-conductive agarose hydrogels as explained afterwards. We chose to work on commercially available human lung epithelial cell lines, the cancerous version of which had been used in our laboratory in different studies. Because normal cells do not grow as well and as fast as cancer cells in culture, the measurement method had to be applicable to small cell volume fractions. However, for convenience in this study, we needed normal cells which can grow rather quickly. That is why the normal cells that we used were actually transformed, i.e. immortalized.

The first objective was to obtain clear and consistent variations in the dielectric properties when cells are present by finding an appropriate matrix in which to embed the cells. The method was developed on cancer cells first. The second objective was to apply exactly the same method on the corresponding normal cells and to compare the results with cancer cells.

The next section describes the materials and experimental methods used in this study. Then, data processing and modelling techniques utilized are presented. This is followed by the obtained results which are finally discussed.

2. Materials and experimental methods

In this study, two cell lines were investigated, one cancerous cell line and a corresponding normal one. For this type of measurement, the cells were embedded in a matrix which was an agarose hydrogel (see section 2.2). For each cell line, seven independent experiments were conducted. Each experiment was carried out on two different volume fractions, the second volume fraction using twice as many cells as the first one. Each experiment involved five gels without cells (matrix), five gels containing the same first volume fraction of cells and five gels containing the same second volume fraction of cells.

The measurement of the matrix is needed to retrieve the properties of the cells thanks to a mixing equation and to evaluate the variation in the dielectric properties when adding the cells. Two volume fractions were used to assess the reliability and consistency of these variations.

2.1. Cell culture

The chosen human lung epithelial cancer cell line was the SK-MES cell line (ECACC, UK), and the normal corresponding one was the NL-20 cell line (ATCC, USA). Both cell lines were cultured in 175 cm^2 cell culture flasks and incubated at 37 °C and 5% CO_2. Each culture vessel contained a complete culture medium and other standard components according to the providers' instructions. The culture medium was mainly composed of high-glucose Dulbecco's Modified Eagle's Medium supplemented by 10% volume of foetal calf serum for

SK-MES cells and of Ham's F-12 Medium supplemented with 4% volume of foetal bovine serum for NL-20 cells.

2.2. Choice and preparation of the embedding matrix

Hydrogels were preferred to aqueous liquids as a matrix to embed the cells because they allowed us to get more stable measurements. Indeed, attempts with liquids showed that the dielectric properties changed quickly with time (already in less than a couple of minutes) because of the sedimentation of the cells. Besides, the model of the open-ended coaxial probe used to retrieve the dielectric properties supposes that the sample is homogeneous. To get the most reliable measurements, we needed a medium in which the spatial distribution of the cells is as homogeneous as possible. Our previous paper (Egot-Lemaire *et al* 2008) explored the feasibility of measuring the dielectric properties of SK-MES cells embedded in collagen gels. One problem was that the collagen gels take about 30 min to set, allowing the cells to sedimentate to some extent. The second problem was that the gels were prepared at physiological ionic strength, which proved to be too conductive to get a good sensitivity and to be able to detect consistent changes in the imaginary part of the complex permittivity.

The two aforementioned problems were solved by using 1.5% w/v agarose gels which have a very quick setting time (less than 2 min) and opting for a low-conductivity, isotonic medium in which the agarose powder is dissolved. Polevaya *et al* (1999) reported that suspending the cells in similar low-conductivity media for 30–45 min did not impair their viability, membrane integrity and ion transport capability. In our case, the total time of handling the cells under those conditions was inferior to that. Thus, we chose to dissolve 1.5% w/v agarose powder in a 5% isotonic glucose solution, whose acidic pH (around 4) was balanced to physiological pH (7.4) conditions by 90 mg L^{-1} of sodium bicarbonate, and buffered by the 1% v/v Hepes buffer. The dissolving of the agarose takes place when bringing the mixture to boil. The gel sets when cooling down at room temperature. To keep the same gel matrix properties for all samples in an experiment, the agarose solution containing dissolved agarose was placed in a water bath at 39 ± 1 °C to keep it in liquid form.

2.3. Dielectric spectroscopy experimental setup and limitations

The experimental setup used in this study was the one presented in Egot-Lemaire *et al* (2008). It allowed us to get good measurement repeatability. Dielectric spectroscopy was performed using a vector network analyser (model 8753E, Agilent Technologies) operated in the frequency range 200 MHz to 2 GHz and connected to a flanged open-ended coaxial probe (Agilent model 85070) via a coaxial cable; 201 linearly spaced frequency points were measured.

Our hydrogel samples needed to be relatively small to achieve a few per cent cell volume fractions, but also needed to have a minimum size. They were laid on the tip of the probe and were thus measured at room temperature (23 ± 1 °C). They fitted the outer diameter of its flange (19 mm); this is the minimum width recommended by the supplier. The commonly estimated sensing depth for this type of probe is at least as big as the outer conductor radius of the probe (Fan *et al* 1990, De Langhe *et al* 1994a, Hoshina *et al* 2001). In our case, this radius is 1.5 mm; we chose a 3 mm thickness for our gel samples in accordance with the sensing depths reported in Semenov *et al* (2000) and Hagl *et al* (2003) dealing with probes of similar dimensions. It also met the supplier's recommendation: in a simple formula related to the permittivity to be measured, the latter gives the minimal thickness of the sample, which yields about 2.3 mm for our samples.

The dielectric properties were calculated from the measurement of the reflection coefficient on the tip of the probe using Agilent 85070E software. The frequency range was chosen within the limits of Agilent's probe model operating range, starting at 200 MHz. This model also supposes that the sample is semi-infinite (i.e. covers a half space) but in practice it is obviously of finite size. This constitutes the first major limitation on the measurements. Especially for small samples, reflections occur at the sample boundaries and could perturb the measured reflection coefficient, thus creating artefacts in the dielectric spectra, as pointed out by several investigators studying the same kind of probe (Grant *et al* 1989, De Langhe *et al* 1994a, 1994b, Sheen and Woodhead 1999, Bao *et al* 1994). Those effects will be well dampened by substantially lossy materials, but poorly by low-loss ones, which is a second limitation of our measurements. These artefacts were attributed to cavity resonance phenomena in Grant *et al* (1989), Sheen and Woodhead (1999) and Bao *et al* (1994). De Langhe *et al* (1994b) showed theoretically that for quite small-diameter samples, reflection coefficients oscillate around the ones obtained for infinite-diameter samples. We chose 2 GHz as the upper frequency to avoid such perturbing effects which were noticeably observed somewhat after 2 GHz on the spectra of our samples.

To assess the effects of the finite size of low-loss gel samples, we conducted the following experiment. Using the same agarose mixture as described in section 2.2, we prepared one gel whose dimensions are representative of our samples described above (19 mm in diameter, 3 mm in height) and three gels of 13 mm in height, being respectively 23 mm, 31 mm and 46 mm in diameter. Figure 1 illustrates the effects of the different sizes of gels on the real and imaginary parts of their permittivities measured from 200 MHz to 6 GHz along with the measurement of 250 mL of deionized water. Below 2 GHz, the imaginary parts of all gels are the same, but the real part of our sample differs from the ones of the bigger gels, which are all roughly the same; however, the real part follows the same decreasing trend with frequency up to the first resonance effect. This means that the measurements performed on our small samples do not yield the 'actual' values of the real part of the permittivity, but only values which are close to the 'actual' values and have a similar behaviour with frequency. As we only want to compare normal and cancer cells in terms of dielectric behaviour and because both normal and cancer cell samples include the same measurement inaccuracies, we do not necessarily need to get the 'actual' values.

2.4. Experimental protocol: outline

The prepared volume fractions were estimated by considering the round-shaped cells as spherical. Their diameters were measured on a slide under a light microscope equipped with a computerized ruler on a sample of 30 cells. The diameter of the SK-MES cancer cells was 18.0 μm (mean) $\pm$ 2.2 μm (standard deviation). The diameter of the NL-20 normal cells was a bit smaller: 15.6 μm (mean) $\pm$ 1.9 μm (standard deviation). After harvesting the cells, they were resuspended in a complete culture medium and their numbers were estimated with the aid of grid-counting chambers (Hycor Kova Glasstic). On seven experiments with each cell line, the prepared volume fractions ranged from 1.2% to 6.1% for the SK-MES cells and from 1.6% to 5.5% for the NL-20 cells, corresponding to, respectively, 4–19 million cells per sample for the SK-MES and 8–27 million cells per sample for the NL-20.

Prior to measurement, the network analyser was calibrated using a standard procedure, in which the standards are air, a short circuit and 250 mL of deionized water. The agarose gels without cells were measured first.

Before starting the experiments, the cells suspended in the culture medium were spun down in a centrifuge at 1200 rpm for 7 min. Then, the supernatant was discarded and carefully

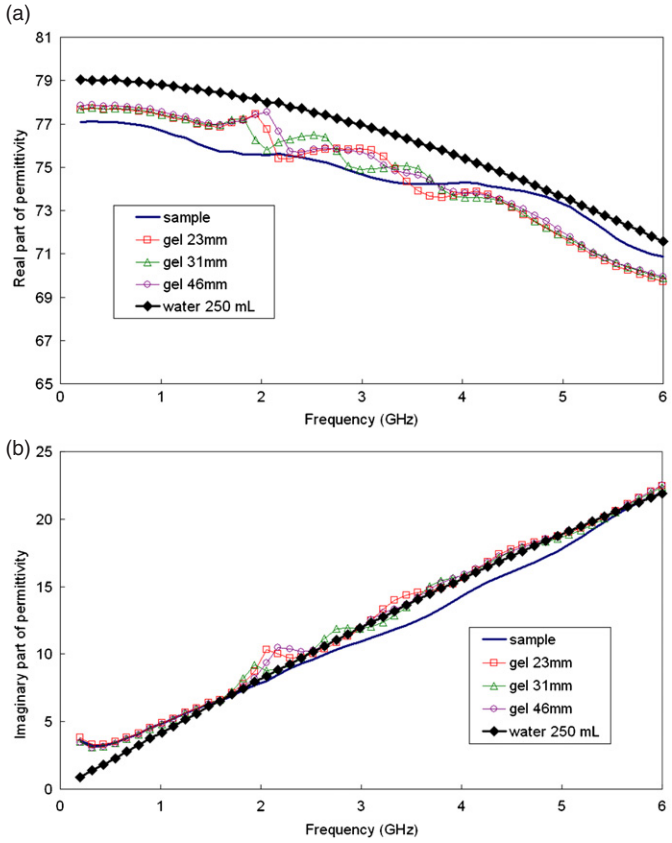

Figure 1. Example of dielectric measurement of gels of different sizes up to 6 GHz. (a) Real part of the complex permittivity. (b) Imaginary part. Solid thick line: 19 mm diameter, 3 mm thickness gel sample; other lines: gels of 13 mm in height; diameters: □ 23 mm, △ 31 mm, ○ 46 mm; ♦ 250 mL of water.

removed as much as possible with a 100 μL pipettor. The cells were resuspended and thus washed once in the solution composed of 5% isotonic glucose, 90 mg L^{-1} sodium bicarbonate and 1% Hepes buffer, and then spun down again at 1200 rpm for 7 min. This allowed us to minimize the influence of the conductive culture medium present within the preceding pellet. Next, the new supernatant was discarded and carefully removed as previously, and the pellet finally mixed homogeneously with 4.9 mL of the agarose liquid solution placed in the water bath. This mixture was used to fill five cylindrical wells with 850 μL each. The gels were allowed to set and to cool down to room temperature for 10 min. They were subsequently measured one by one, seven times each, thereby checking the good stability and repeatability of the measurements.

3. Analysis and processing of experimental data

Prior to further processing the experimental data, the noise produced by the network analyser and its drift over time was first removed from the raw data by the efficient curve smoothing technique implemented by the Savitsky-Golay filter. This filter is a built-in function within the software Matlab (The MathWorks Inc.).

3.1. Analysis of the variation of the measured complex permittivity

To compare the dielectric behaviour of normal and cancer cells, we calculated the absolute variation $\Delta\varepsilon'$ and $\Delta\varepsilon''$ of, respectively, the real and imaginary parts of the measured complex permittivity when cells are present, relatively to the properties of the matrix alone (gel without cells). We noted that this absolute variation was proportional to the volume fractions used. Thus it can be written as

$$\Delta\varepsilon = \varepsilon_{\text{eff}} - \varepsilon_m = k\Phi \tag{1}$$

where ε_m is the complex permittivity of the matrix (gels without cells), ε_{eff} is that of the composites (gels with cells), Φ is the cell volume fraction and k is the proportionality factor. By using the results of the two volume fractions per experiment, k was calculated by linear regression in the least square sense for each measured frequency point. One value (k') was obtained for $\Delta\varepsilon'$ and another (k'') for $\Delta\varepsilon''$.

Because normal and cancer cell samples were measured under the same conditions, this can give us a first idea whether the measurements are statistically the same or not for both cell lines, independently of the limitations of the measurement method. This gives us a first means to compare normal and cancer cells in terms of dielectric properties of the composites, but not to compare the dielectric properties of the cells themselves. To do this, we need to apply a dielectric model to the cells.

3.2. Method used for cell modelling and measurement fitting

An effective medium approximation was used to describe the effective complex permittivity of the composite gels as a function of that of the matrix and that of the cells. The Maxwell–Wagner equation was applied. It had been derived to describe a two-phase medium with spherical inclusions not interacting with each other, i.e. a dilute suspension, practically for a volume fraction below 0.1. Originally formulated for non-conducting particles in an insulating medium (Bonincontro *et al* 1980), it was extended to complex permittivity by Pauly and Schwan (Bordi *et al* 2002), and some authors have even used it at 10 GHz (Bonincontro *et al* 1983). Equation (2) gives the expression of the Maxwell–Wagner equation:

$$\frac{\varepsilon_{\text{eff}} - \varepsilon_m}{\varepsilon_{\text{eff}} + 2\varepsilon_m} = \Phi\frac{\varepsilon_{\text{cell}} - \varepsilon_m}{\varepsilon_{\text{cell}} + 2\varepsilon_m} \tag{2}$$

where $\varepsilon_{\text{cell}}$ is the complex permittivity of a cell and Φ is the cell volume fraction. Here the use of this equation is consistent with equation (1), i.e. the fact that the variation in permittivity when cells are present was found proportional to the volume fraction.

As the volume fraction is only estimated, a better approximation of it was obtained by allowing it to vary when fitting the measurements of ε_{eff} with equation (2). The dielectric spectra of a eukaryotic cell are described by dielectric dispersions extending over a wide frequency range, from the low frequencies to the lower part of the UHF range (Ermolina *et al* 2000, Asami 2002). The complete double-shell model is well described by the sum of two Debye-type dispersions and a conductivity term. We applied equation (2) to calculate $\varepsilon_{\text{cell}}$ using our measurements ε_m and ε_{eff} on each cell line and the prepared volume fractions. Because of the narrowness of the explored UHF range, the obtained curves did not reveal a complete Debye-type relaxation, but they only somehow exhibited an end of relaxation tail. Consequently it is not reasonable to try to determine, let alone to fit all the parameters of a Debye-type relaxation, since they would be too inaccurate. The real part of $\varepsilon_{\text{cell}}$ did not exhibit big variations and its imaginary part showed a variation rather inversely proportional to the frequency. Therefore, our assumption was to approximate the permittivity of a cell

$\varepsilon'_{\text{cell}}$ as a constant and its conductivity σ_{cell} also as a constant on our narrow frequency range. So our model cell did not take into account any cell compartment, but considered the cell as a 'homogeneous' particle. This model is not perfect, but as it produced a satisfactory fit of the measurements (see section 4), it was chosen to fit the experimental data. Because of the limitations in the measurement method and in this approximate modelling approach, it is not expected that 'actual' values will be obtained, but only an approximation of them on a very small frequency range (200 MHz to 2GHz). However, this modelling approach is a means of comparison between normal and cancer cells on top of the one explained in section 3.1.

The fitting process applied a complex nonlinear least-square procedure using the trust-region reflective-Newton algorithm implemented with Matlab. The real and imaginary parts of the measured complex permittivity ε_{eff} were fitted simultaneously. The volume fraction was allowed to vary (within 30% of its prepared value, taken as the initial value in the fitting) to fit the curves obtained with both volume fractions tested per experiment. This was done for each experiment on each cell line to compare normal and cancer cells.

3.3. Statistical method used

For each experiment, either on the SK-MES or on the NL-20 cells, we calculated

- k (actually k' and k'') of equation (1) as a function of frequency, for the absolute variations in the measured complex permittivity;
- a constant cell relative permittivity $\varepsilon'_{\text{cell}}$ and a constant cell conductivity σ_{cell} fitting the measurements.

For each cell line, these results were obtained in seven experiments. The mean and standard deviation values were calculated in these seven experiments. Finally, to compare the two cell lines, the p-values were calculated using the statistical two-tailed Welch's t-test. It takes into account a potential difference between the standard deviations obtained in the SK-MES and in the NL-20 cells.

4. Results

4.1. Typical dielectric measurement result

Figure 2 shows a typical example of measurement result for one experiment, for both ε' and ε''. Means along with standard deviations, all calculated on five gels, are represented for the matrix and the composites gels containing cells, for both cell volume fractions tested.

Both cell lines exhibited the same behaviour in terms of variation of dielectric properties when cells are present in gels compared to the matrix without cells. When the cell volume fraction increased, the real part ε' of the complex permittivity consistently decreased in the whole explored frequency range. The decrease was higher for higher frequencies. The imaginary part ε'' consistently increased with the cell volume fraction, in the whole frequency range, but the increase was noticeably higher at lower frequencies.

4.2. Detectability of cells

The measurement reproducibility from one gel to another was assessed for each volume fraction tested by calculating the fractional error (standard deviation divided by the mean, calculated on five gels) as a function of frequency. At the very most, it reached 0.5% for ε' and 5% for ε''. The fractional error of ε'' was higher in the lower part of the explored frequency range (where ε'' is particularly low though), but was always lower than 1% after 1 GHz.

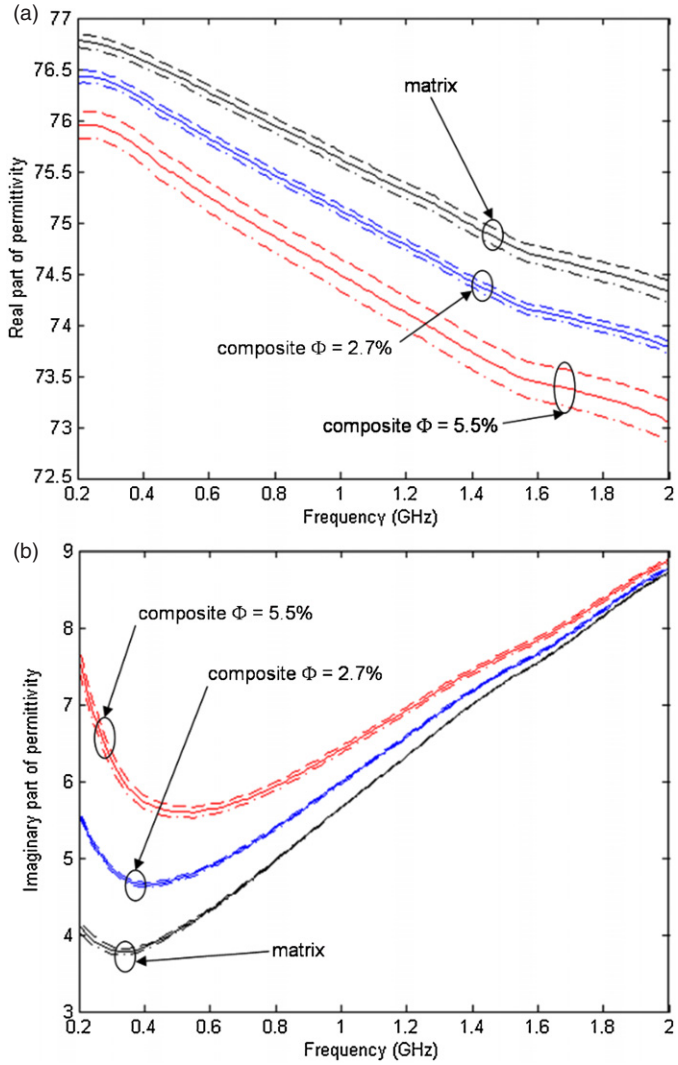

Figure 2. Example of measurements of the matrix alone and composites gels for NL-20 cells on two volumes fractions Φ (2.7 and 5.5%): means (solid line) plus (dashed lines) and minus (dash-dotted lines) standard deviations. (a) Real part of the complex permittivity. (b) Imaginary part.

Besides, the difference between the groups (matrix alone, first and second volume fractions) is statistically significant ($p < 5\%$). We consider that the minimal cell volume fraction detectable by our method is about 1% for both cell lines, corresponding to about 5 million NL-20 normal cells per sample (i.e. 5.9 million per mL) and 3.2 million malignant SK-MES cells per sample (i.e. 3.8 million per mL).

4.3. Curve fitting

As mentioned earlier, the fitting of the measurements by a constant cell permittivity and constant cell conductivity applied in the Maxwell–Wagner equation gave satisfactory results.

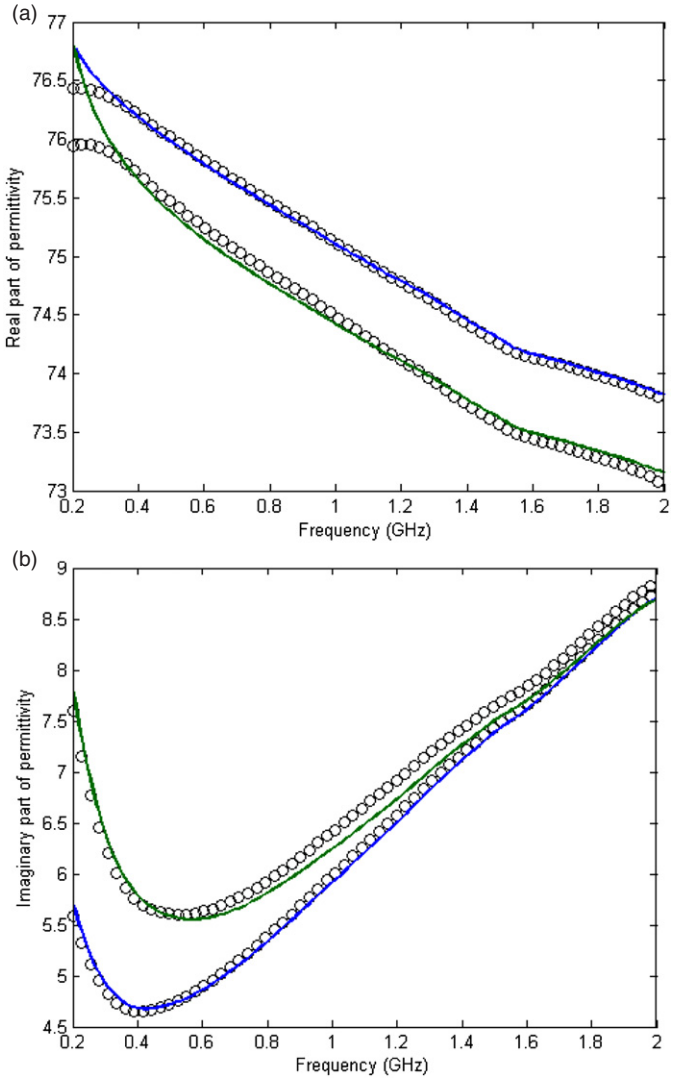

Figure 3. Fitting the measurements of the composites by modelling a cell with constant permittivity and conductivity. (a) Real part. (b) Imaginary part. o: measured data; solid line: fitting curves.

Figure 3 illustrates how ε_{eff} measurements shown in figure 2 are fitted by this approximate model. The fitting is not perfect for the real part of ε_{eff} at the low end of the explored frequency range. However, the imaginary part is very decently fitted, especially in the low part of the frequency range. This was the best compromise we could find to fit the set of experimental data. In some studies dealing with dielectric properties published so far, fittings of relaxations are often satisfactory but not always perfect either on some bits of the frequency range considered. On all fitting procedures, the difference between the initial and fitting cell volume fractions was typically 20% for SK-MES cells and 23% for NL-20 cells on average. It is of the same order of magnitude and is consistent with the close behaviour observed between the two cell lines (see below).

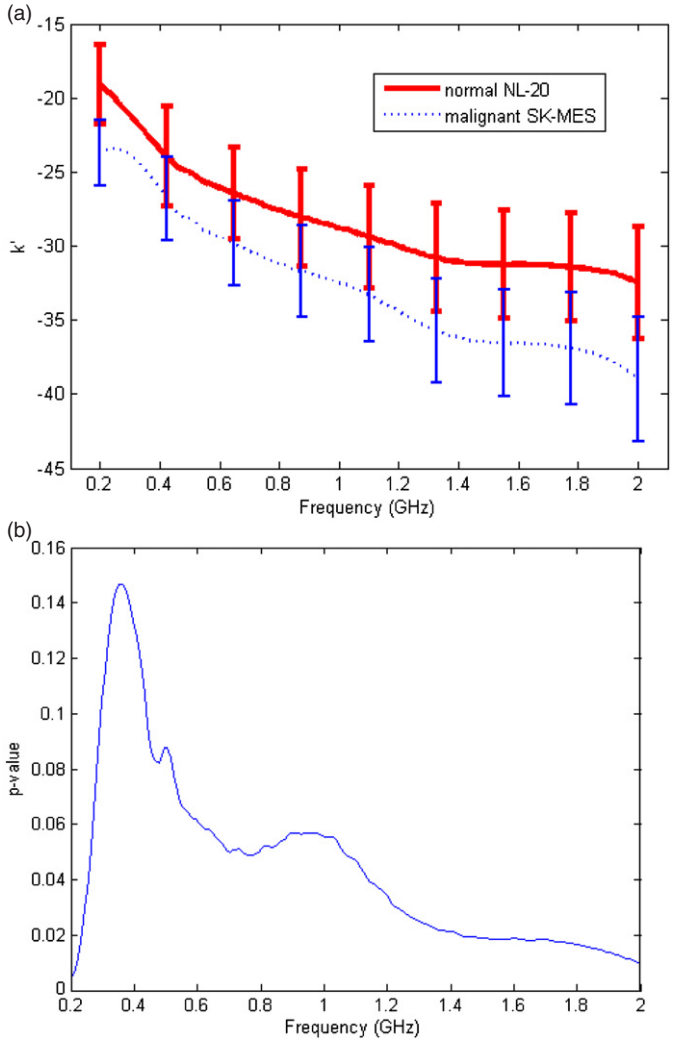

Figure 4. Coefficient k' as a function of frequency. (a) Comparison between NL-20 (solid line) and SK-MES (dashed line): means given with standard deviation error bars. (b) p-values resulting from the comparison in (a).

4.4. Comparison between the two cell lines

4.4.1. Variations of the measured complex permittivity. The real k' and imaginary k'' parts of the absolute variations $\Delta\varepsilon$ on both cell lines are plotted respectively in figures 4 and 5 along with the p-values resulting from the comparison between the behaviours of the normal and malignant cells.

The results presented in figure 4 show that there is a slight difference between the real parts of the composite gels of normal and cancer cells. They tend to demonstrate that the variations in the measured real parts of the composites of SK-MES and NL-20 cells are statistically significant only after 1.1 GHz ($p < 5\%$), and also below 300 MHz, but less significant between 300 MHz and 1.1 GHz.

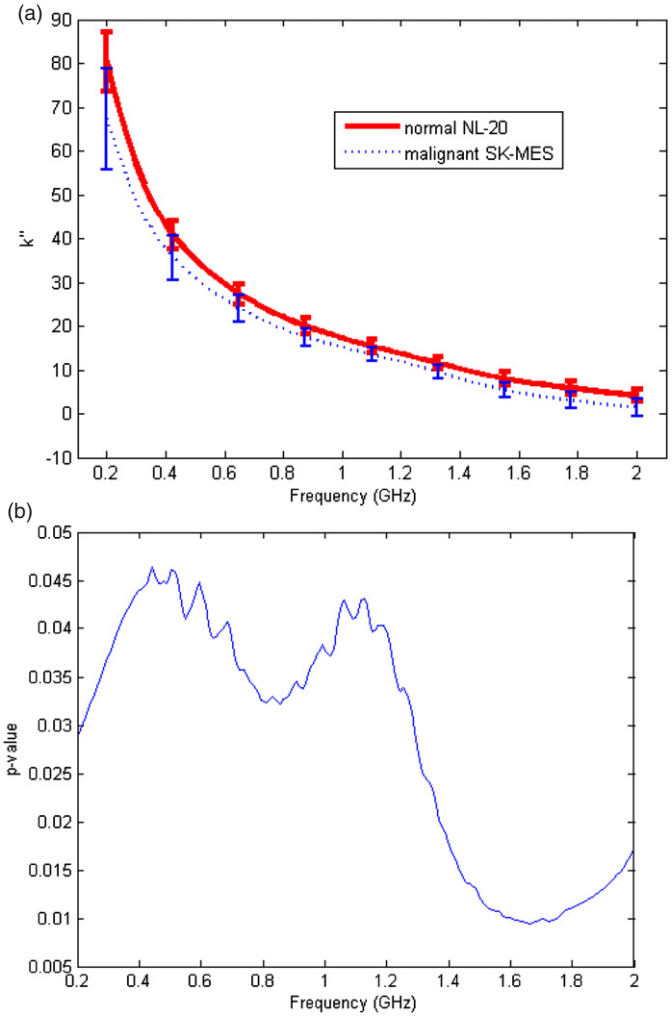

Figure 5. Coefficient k'' as a function of frequency. (a) Comparison between NL-20 (solid line) and SK-MES (dashed line): means given with standard deviation error bars. (b) p-values resulting from the comparison in (a).

The results presented in figure 5 show that there is also a slight difference between the imaginary parts of the composite gels of normal and cancer cells and that this difference is statistically significant ($p < 5\%$) in the whole frequency range 200 MHz to 2 GHz.

Even if k' and k'' do not represent the dielectric properties of the cells, their behaviours show that there is a detectable difference between the two cell lines, especially regarding the imaginary parts of the measured composite gels. This result does not involve modelling of the cells or fitting.

4.4.2. Fitting constant permittivity and constant conductivity. The constant permittivity and conductivity which model the cells were retrieved for each of the seven experiments for both cells lines. Tables 1 and 2 present the statistical results obtained on respectively the constant

Table 1. Fitting constant permittivity of cells calculated in seven experiments.

	Mean	Standard deviation	p-value
Normal NL-20	48.1	2.7	0.08
Malignant SK-MES	45.6	2.1	

Table 2. Fitting constant conductivity of cells calculated in seven experiments.

	Mean (S m^{-1})	Standard deviation (S m^{-1})	p-value
Normal NL-20	0.89	0.06	0.01
Malignant SK-MES	0.77	0.08	

permittivity and the constant conductivity, in terms of means and standard deviations for each cell line.

According to the results presented in table 1, the difference between the permittivities of SK-MES and NL-20 cells could be considered as 'borderline statistically significant', because the p-value is between 5% and 10%, in line with the classification described in Polevaya *et al* (1999). Nevertheless, the difference in permittivity between the two cell lines appears to be quite small.

According to the results presented in table 2, the difference between the conductivities of SK-MES and NL-20 cells could be considered as statistically significant, as the p-value is 1%. Then again, the difference in conductivity between the two cell lines appears to be rather small.

These results obtained from modelling confirm that there is a slight difference in dielectric properties between the NL-20 normal cell line and the SK-MES cancer cell line in the UHF range for both the permittivity and conductivity of the cells, the difference being especially statistically significant for the conductivity.

5. Discussion

5.1. Comparison with results obtained on lung tissues

In section 1 we mentioned the significance in understanding the difference between the dielectric properties of tissues and cells, especially in the UHF range. The cells we used in this study are lung epithelial cells which line the bronchi. Several papers report the dielectric properties of lung tissue, either measured on humans or animals, at room temperature or brought to body temperature; they were made *ex vivo*. Thus, a comparison of those results with ours has to be considered with care. We found no dielectric spectroscopy study of *in vivo* lungs parts where our cell lines were taken from. However, it is interesting to compare the tendencies of these studies with ours.

The dielectric properties are most often found on healthy lung tissue for different frequencies. Durney *et al* (1986) as well as Foster and Schwan (1996) give a relative permittivity of about 35 and a conductivity of 0.53–0.73 S m^{-1} of human *ex vivo* lung tissues between 200 MHz and 1 GHz (at room temperature). Stuchly and Stuchly (1980) report similar permittivity values, and a conductivity ranging from 0.62 to 0.72 S m^{-1} between

200 and 500 MHz (human lungs *in vitro* at body temperature). The most relevant study on lungs (Joines *et al* 1994) reports values for healthy and malignant lung tissues at room temperature between 50 MHz and 900 MHz. Their results show that the permittivity of normal lung tissue is higher than that of the malignant one: the permittivity of normal (respectively malignant) lungs was found to decrease from 65.0 to 60.3 (respectively from 61.0 to 54.0) between 200 MHz and 900 MHz. We found the same tendency between normal and malignant cells in the present study. In Joines *et al* (1994) the conductivity of normal lung tissue was found lower than that of the malignant one: the conductivity of normal (respectively malignant) lungs increased from 0.64 S m^{-1} to 1.16 S m^{-1} (respectively from 0.84 S m^{-1} to 1.24 S m^{-1}). This is the opposite tendency as the one of our study on cells. It could mean that either or both of the extracellular matrix and blood supply play the major role in the conductivity of lung tissues at these frequencies, or that the dielectric behaviour at tissue cell volume fractions is different than at low volume fractions.

5.2. Comparison with other studies on normal and cancer cells

The most comprehensive study comparing the dielectric properties of normal and malignant cells was done on B-cells and T-cells (Polevaya *et al* 1999, Ermolina *et al* 2001) and covered a frequency range from 200 kHz to 3 GHz. The investigators modelled a cell as a double shell to fit their experimental data. The permittivities of the cytoplasm and nucleus were needed to be fixed for the fitting. Their changes within a realistic range had previously been shown not to affect much the dielectric properties of the suspension. The six fitted parameters were the permittivities of the cell membrane and nuclear envelope, the conductivities of the cell membrane, nuclear envelope as well as of the cytoplasm and nucleus. The latter two parameters are quite influent in determining the high frequency part of the dielectric spectrum of a cell (Ermolina *et al* 2000). Their difference in normal and malignant T-cells was not found statistically significant. But the other four ones were higher for normal than malignant T-cells, and all six parameters were found higher for normal B-cells compared with malignant B-cells; all these results were statistically significant. However, in the same study, the authors also investigated the transformed Malaga B-cell line (immortalized by virus transfection), which is a similar case to our NL-20 cells. The conductivities of the membrane, as well as cytoplasm and nucleus of these Malaga cells, were found to be more in the value range of cancer B-cells (even with no statistical difference) than normal B-cells. This feature could help to understand that we have found that conductivities of SK-MES and NL-20 cells are quite close to each other. Indeed the authors interpreted these three conductivities as representative of the common immanent dividing feature of cancer and transformed cell lines. It would be relevant to investigate whether non-dividing normal lung cell lines produce higher differences than those we found between NL-20 and SK-MES cells.

Nonetheless, our study in the UHF range finds the same kind of tendency as in the aforementioned study concerning normal versus malignant cells, namely higher dielectric properties for normal cells than for cancer cells. This behaviour actually appears as the opposite behaviour of cancer versus malignant tissues already observed in this frequency range except for lungs and kidney (see section 5.1; Joines *et al* (1994)). In the microwave frequencies, the dielectric behaviour of tissues is greatly influenced by the tissue water content (Pethig and Kell 1987). Tumour tissues are said to have significantly higher water and sodium content than homologous normal tissues (Foster and Schwan 1996); that is why they are often said to be distinguishable in terms of dielectric properties and expected to exhibit somewhat larger permittivity and conductivity values than homologous normal tissues. This result is vastly found, e.g. for breast tissue (Sha *et al* 2002, Chaudhary *et al* 1984), but not for certain

organs highly perfused with blood such as the spleen or kidney (Foster and Schwan 1996, O'Rourke *et al* 2007).

More studies should be carried out on cells to investigate further these discrepancies between cells and tissues. Pethig and Kell (1987) explain that dielectric behaviours applicable for diluted cell suspensions are not valid for high cell volume fraction tissues. Therefore, it is not necessarily easy to compare both behaviours. They could mainly be explained either by the predominance of the effect of extracellular matrix and blood supply on the dielectric properties of the tissue, or by the dead or living state of the considered tissue, which can tie up with the blood perfusion matter.

6. Conclusion

In this paper we investigated the dielectric behaviours of the human lung cancer SK-MES cell line and compared them to those of the corresponding normal NL-20 cell line in the frequency range 200 MHz to 2 GHz. We found consistent dielectric changes with increasing cell volume fractions in agarose hydrogels. The method proved to be quite sensitive as the smallest detectable cell volume fraction was about 1%.

By comparing the variation in dielectric properties of composites gels, we found a slight difference between the normal and malignant cell lines, which was statistically significant mainly for the imaginary part. This small difference between the two cell lines was confirmed by using an approximate model of the cells. The difference in conductivity of normal and malignant cells was found statistically significant. Both permittivity and conductivity were found to be slightly higher for the NL-20 normal cells than for the SK-MES cancer cells. This behaviour is rather different of what is often observed in cancer tissues when compared to normal tissues. However, it is quite in agreement with another study which compared normal versus malignant white blood cell lines.

Actually the NL-20 is an immortalized normal cell line, so a non-dividing, purely normal lung cell line should be investigated in the future to see if the differences in the SK-MES cancer cells are higher or lower than the ones observed here. More microwave dielectric spectroscopy experiments of human lung tissue samples would also be desirable to better understand the contribution of cells in the dielectric properties of tissues in the UHF range.

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