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# Measurements of the dielectric properties of peripheral blood mononuclear cells and trophoblast cells using AC electrokinetic techniques

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**Abstract**

The separation of trophoblast cells from the maternal circulation could provide a valuable diagnostic tool for prenatal diagnosis of genetic abnormalities. This has been attempted using antibody methods, but due to non-specificity of the antibodies, maternal cell contamination remains a problem. We have investigated the potential of dielectrophoretic separation methods as a means of isolating trophoblast cells from mixed peripheral blood mononuclear cells. To determine the potential of this method the dielectric properties of trophoblast cells and mixed peripheral blood mononuclear cells were measured using dielectrophoretic crossover and single cell electrorotation methods. Both dielectrophoretic crossover data and electrorotation data gave an average specific membrane capacitance of the peripheral blood mononuclear cells of 11.5 mF $m^{-2}$. Trophoblast cells prepared using three different methods had a higher average specific membrane capacitance in the range 13–18 mF $m^{-2}$. The differences in capacitance between the cell types could be exploited as the basis of an AC electrokinetic-based system for the separation of trophoblast cells from peripheral blood mononuclear cells. 

*Keywords:* Trophoblast; Dielectrophoresis; Electrorotation; Prenatal diagnosis; Cell separation

## 1. Introduction

The placenta is a vital tissue involved in supporting the growth of the foetus by providing nutrients and removing metabolites. It has evolved to fulfil this function by being bathed in maternal blood that provides nutrients and carries away metabolites. Structurally it is organised into a central core containing foetal blood vessels and peripherally trophoblast cells. The trophoblast is made up of an inner layer of mononuclear cytotrophoblast cells that proliferate and differentiate to form the outer layer of terminally differentiated syncytiotrophoblast cells. The latter are multinucleated and form by fusion of the cytotrophoblast cells. Syncytiotrophoblast cells are shed continuously into the maternal circulation in measurable amounts (100 000 cells per day) and lodge in the capillary beds of the maternal lungs where they are degraded [1]. In early pregnancy, the maternal spiral arterioles are invaded by specialised cytotrophoblast cells and this is essential for the develop-

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ment of a good blood supply for the placenta [2]. There is therefore the propensity for a heterogeneous population of trophoblast cells to be present in the maternal bloodstream at different gestations.

Non-invasive methods for retrieving trophoblast or foetal blood cells from the maternal circulation for the diagnosis of genetic abnormalities have been developed [1]. Despite advances in molecular biological techniques, attempts to isolate the cells have been confounded by contamination of the samples with maternal cells, making diagnosis less reliable than invasive techniques such as amniocentesis and chorionic villous sampling. These techniques, while more reliable, carry a small but significant risk of miscarriage. To date all separation strategies have been aimed at antibody based techniques, which identify sub-populations of rare foetal or placental cells and are limited by the specificity of the antibodies used. In order to advance non-invasive prenatal diagnosis, a non-antibody based method is required to identify and separate trophoblast cells from maternal blood. The aim of this work was to determine whether there were differences in the dielectrophoretic (DEP) properties of peripheral blood mononuclear cells (over 80% of which are lymphocytes) and trophoblast cells in anticipation of the development of a novel method for sorting trophoblast from maternal blood.

AC electrokinetic-based cell sorting is a new technology that separates sub-populations of cells according to their DEP properties. [3–8]. The dielectric properties of individual cells within sub-populations are measured using AC electrokinetic methods and if these differences are sufficient, then the cell sub-populations can be separated. This method has been used successfully to separate human breast cancer cells (MDA 231) from T-lymphocytes [3], and HL60 human leukaemia cells from blood [4].

## 2. Theory

AC electrokinetics is the term used to describe the interaction of AC electric fields with particles such as cells, and the general theory governing the behaviour of particles can be found in a number of books and review articles (e.g. [9,10]). In this paper, both DEP crossover frequency measurements and electrorotation (ROT) spectra were used to characterise the dielectric properties of human peripheral blood mononuclear cells (PBMCs) and trophoblast cells.

DEP crossover frequencies were measured as a function of suspending medium conductivity and the data analysed according to the method of Huang et al. [8]. The crossover frequency can be written as a function of membrane capacitance and membrane conductance according to:

$$f_{\mathrm{Cross}} = \frac{\sqrt{2}}{8\ \pi r C_{\mathrm{Mem}}}\sqrt{(4\ \sigma_m - rG_{\mathrm{Mem}})^2 - 9\ r^2 G_{\mathrm{Mem}}^2} \quad (1)$$

where $G_{\mathrm{Mem}}$ is the specific conductance of the membrane, $C_{\mathrm{Mem}}$ the specific membrane capacitance and $\sigma_{\mathrm{m}}$ the conductivity of the suspending medium. The data were analysed by minimising the error between the measured and theoretically determined data. In this way an average measurement of the conductance and capacitance of the membrane for a collection of similar cells was obtained. For each data set the confidence of the fit was determined by calculating a regression coefficient given by:

$$\rho = 1 - \left[\frac{\sum_i (X_{\mathrm{expt}}(\omega_i) - X_{\mathrm{est}}(\omega_i))^2}{\sum_i (X_{\mathrm{expt}}(\omega_i))^2}\right] \quad (2)$$

The dielectric parameters of cells were obtained from rotation data by fitting the data to the shell model [11]. Owing to the limited frequency bandwidth use in our experiments the single shell model was used. This models the cell as a homogeneous solid sphere (consisting of the cytoplasm, including the nucleus and nuclear membrane) surrounded by the cell membrane. Although this is a simplification of the actual situation, the model has been found to be reasonably effective in predicting the dielectric properties of cells.

## 3. Materials and methods

All blood and placental samples were obtained following informed written consent as approved by the Yorkhill Ethics Committee.

### 3.1. Peripheral blood mononuclear cells (PBMCs)

PBMCs were prepared from whole blood diluted

1:2 with PBS and underlayered with 5 ml Histopaque 1077 (Sigma). The cells were centrifuged at $320 \times g$ for 20 min at room temperature and the buffy coat was aspirated. Individual cells from this preparation were used for both DEP crossover and ROT measurements.

### 3.2. Trophoblast cells

#### 3.2.1. Preparation from whole placenta

Normal term placentas were obtained immediately after delivery by Caesarean section or spontaneous vaginal delivery. Trophoblast cells were prepared according to a modified protocol taken from Kliman et al. 1986 [12]. Several cotyledons were removed from the underlying fibrous elements and placed in Puck's saline (GIBCO BRL) with 25 mM HEPES (Sigma). The tissue was divided with a scalpel to expose the maximal surface to the medium, and incubated in Dulbecco's modified Eagle's medium containing 25 mM HEPES (Sigma), 5% foetal calf serum and 1 mg/ml collagenase type IV. The tissue was incubated at 37°C for 30 min and agitated gently every 5 min. The supernatant was removed and run on a Histopaque 1077 gradient. The cells collected from the interface were removed and washed in Puck's saline by centrifugation for 10 min at 2000 RPM. They were then resuspended in Puck's medium. Cell viability was verified by Giemsa staining and the percentage of trophoblast cells in the final preparation evaluated by flow cytometry of trophoblast specific cytoskeletal antibody (JMB2) labelled cells [13].

#### 3.2.2. Preparation of cytotrophoblast from amniochorion

Term placentas were obtained and the amniochorion removed and enzymatically digested according to the method described by Shorter et al. 1990 [14]. Cell viability was verified by Giemsa staining and the percentage of trophoblast cells in the final preparation evaluated by flow cytometry of trophoblast specific cytoskeletal antibody (JMB2) labelled cells [13].

### 3.3. Retroplacental blood preparation

The preparation of retroplacental blood was carried out as described by Johansen et al. 1994 [13]. Term placentas from normal births were obtained within 15 min of delivery and processed immediately. Retroplacental blood was collected by making 15–20 cuts (each 1–2 cm deep) with a scalpel blade into the chorion. The umbilical cord was clamped and the retroplacental blood was collected by squeezing the placenta by hand. The blood was filtered twice through 100 μm gauze to remove clots and tissue fragments and made up to a final volume of 12 ml with PBS/0.015 M EDTA. A 10 ml aliquot was run on a histopaque gradient and the buffy coat was collected. The final sample contained PBMCs, trophoblast cells and some erythrocytes. Small numbers of multinuclear trophoblast cells could be easily identified and the DEP characteristics of these particular cells were measured.

### 3.4. AC electrokinetic measurements

Dielectrophoresis and ROT measurements were performed using electrodes of the hyperbolic polynomial design [15], fabricated using conventional photolithographic methods. The electrodes consisted of a gold/palladium/titanium sandwich, 100 nm thick, fabricated on glass microscope slides. Electrodes had dimensions of 500 μm between opposite tips. The electrodes were energised phase sequentially using a direct digital synthesis four phase oscillator operating at a frequency of up to 20 MHz with an applied voltage of 5 V peak to peak. For the measurements, cells were harvested, washed in PBS then resuspended in an appropriate conductivity of isoosmotic sucrose/glucose (9% w/v sucrose/0.1% w/v glucose) in phosphate buffer with 0.1 mM EDTA and 0.8% w/v BSA. The final pH was between 6.8 and 7.2. An aliquot of the cell suspension was pipetted onto the electrode array at an appropriate concentration so that no more than 10–20 cells were present in the electrode array during an experiment. Both DEP and ROT data were obtained using medium conductivities in the range 25 to 60 mS $m^{-1}$. The medium conductivity was measured using a conductivity meter (RS Components Model No 180-7127). Cell viability was maintained by controlling osmolality accurately by the addition of sucrose. Osmolality was measured with a Gonotech Osmomat 030 meter. Experiments were performed at 20°C

and cells were observed using an Olympus microscope in phase contrast.

### 3.4.1. Dielectrophoresis crossover frequency measurements

The DEP crossover frequency of the cells was measured by observing the motion of the cells as a function of frequency at or near the crossover point. Two phase sinusoidal signals were applied to alternate electrodes of the polynomial electrode at a potential of 5 V peak to peak. Cells were pipetted onto the electrode chamber and a cover slip was placed over the electrode assembly. Motion of the cell in response to the applied field was observed using a microscope. At low frequencies, cells experienced positive DEP and moved towards the electrode edge, whilst at higher frequencies, cells were repelled away from the electrode edge. The crossover frequency was ascertained by observing the motion of cells within 10–20 μm of the electrode edge. Measurements were made at four conductivities, 49, 63, 76 and 91 mS $m^{-1}$ and 15–20 cells were measured at each conductivity. Only cells with intact membranes were selected for analysis. Multinuclear trophoblast cells were selected where ever possible to minimise the possibility of PBMCs being analysed.

### 3.4.2. ROT spectra

The rotation data of cells were recorded on S-VHS video for further analysis. Typically nine points per decade were recorded over the frequency range 1–20 MHz and a complete spectrum was obtained in 20–30 min. Only cells residing within the central area of the electrode chamber were measured. The rotation rate was determined by analysing five complete rotation cycles of individual cells with a stopwatch.

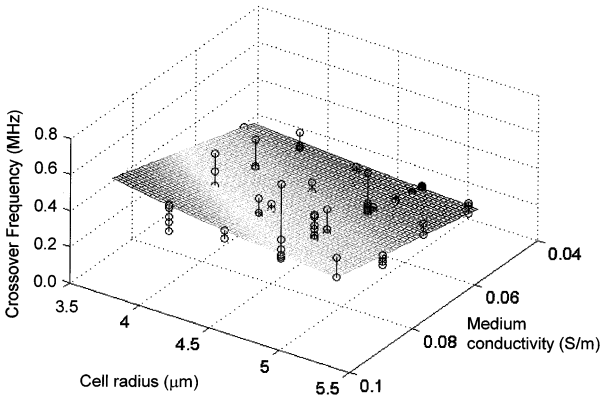


Fig. 1. A plot showing the DEP crossover frequency for human PBMCs from male subjects plotted as a function of medium conductivity and cell radius. The contour represents the best fit to Eq. 1 with $C_{Mem} = 11.5$ mF $m^{-2}$ and $G_{Mem} = 180$ S $m^{-2}$.

## 4. Results

### 4.1. DEP crossover measurements

#### 4.1.1. PBMCs

*4.1.1.1. Male volunteers.* The DEP crossover frequency for PBMCs obtained from male subjects is shown in Fig. 1. The crossover data are plotted as a function of medium conductivity and cell radius. Also shown in the figure is the contour that represents the best fit to Eq. 1. The crossover frequencies of 149 cells were measured and the data points are shown on the graph (where possible). Many cells of the same radius exhibited a similar crossover frequency so that the data points are superimposed. The best fit to the data gave a mean specific membrane capacitance, $C_{Mem} = 11.5$ mF $m^{-2}$ for PBMCs with a specific membrane conductance, $G_{Mem} = 180$ S $m^{-2}$. The regression for the data was $\rho = 0.965$.

*4.1.1.2. Female volunteers.* The crossover frequency for PBMCs obtained from both pregnant (84 cells) and non-pregnant women (82 cells) was

Table 1
Summary of the dielectric properties of PBMCs as determined from DEP crossover measurements

| Cell type | Number of cells analysed | Specific membrane capacitance (mF $m^{-2}$) | Specific membrane conductance (S $m^{-2}$) | Regression coefficient |
|---|---|---|---|---|
| Male PBMCs | 149 | 11.5 | 180 | 0.965 |
| Non-pregnant female PBMCs | 82 | 11.6 | 3500 | 0.985 |
| Pregnant female PBMCs | 84 | 11.5 | 1300 | 0.969 |

also measured (data not shown) and the data fitted to Eq. 1. The results were very similar to those obtained for PBMCs obtained from men. A summary of the data is shown in Table 1.

#### 4.1.2. Trophoblast cells

*4.1.2.1. Cytotrophoblast from amniochorion.* The DEP crossover frequency of trophoblast cells derived from the amniochorion was measured at the same suspending medium conductivities used for the PBMC measurements. 77 individual cells were analysed from 20 placental preparations. The size of the cells varied markedly from 13 to over 30 μm in diameter with a mean of 17.7 μm and standard deviation of 3.3 μm.

A plot of the DEP crossover frequency as a function of cell radius and medium conductivity is shown in Fig. 2. There is a much greater spread in the range of crossover frequencies than was measured for the PBMCs. The best fit to Eq. 1 was obtained with a mean specific membrane capacitance, $C_{\mathrm{Mem}} = 18.2$ mF m$^{-2}$ and a membrane conductance $G_{\mathrm{Mem}} = 3.3 \times 10^3$ S m$^{-2}$. In contrast to the PBMC data, the regression in this case was lower with $\rho = 0.72$, indicating that cells were heterogeneous with a wide range in both capacitance and conductance. Analysis of the data for the these cells was also made by forcing $G_{\mathrm{Mem}} = 0$. This gave the same value of membrane capacitance (18.2 mF m$^{-2}$) but with a lower regression at 0.48.

*4.1.2.2. Trophoblast from whole placenta.* The crossover frequency for 14 cells enzymatically isolated from whole placenta was measured over the same range of conductivities. The cells were in the range 12–40 μm in diameter with a mean of 20.0 μm and standard deviation of 10.6 μm.

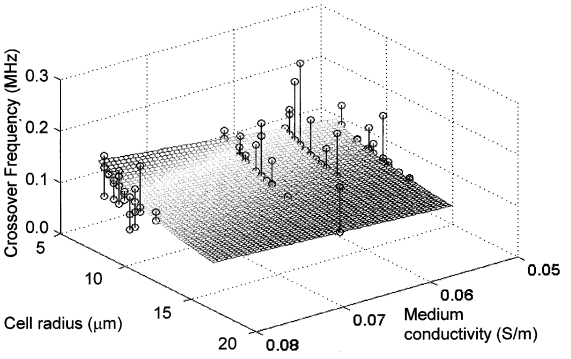


Fig. 2. A plot of the DEP crossover frequency as a function of cell radius and medium conductivity for cytotrophoblast cells derived from amniochorion. The contour represents the best fit to Eq. 1 with a mean specific membrane capacitance, $C_{\mathrm{Mem}} = 18.2$ mF m$^{-2}$ and $G_{\mathrm{Mem}} = 3300$ S m$^{-2}$.

The crossover frequency for these cells was recorded at two medium conductivities and a simpler approach was used to analyse the data. For low frequencies, and assuming that the particle has zero membrane conductivity (from Eq. 1 with $G_{\mathrm{Mem}} = 0$), the specific membrane capacitance is given by the following expression:

$$C_{\mathrm{Mem}} = \frac{\sigma_{\mathrm{m}}}{\pi\sqrt{2} f_{\mathrm{Cross}} r} \tag{3}$$

Analysis of the crossover frequencies of the data using this expression gave a wide range of specific membrane capacitances covering a range from 7.7 to 33.8 mF m$^{-2}$. The mean was 14.5 mF m$^{-2}$ with a standard deviation of ± 7.4 mF m$^{-2}$.

*4.1.2.3. Retroplacental trophoblast cells.* The DEP crossover frequency of 14 individual trophoblast cells obtained from retroplacental blood was measured. Eleven of the cells were 12–16 μm in diameter. All 14 cells were analysed in a single medium conductivity (82 mS m$^{-1}$) so that the data were fitted

Table 2
Summary of the average values for the membrane capacitance and conductance for all trophoblast cells calculated from DEP crossover data

| Cell type | Number of cells analysed | Membrane capacitance (mF m$^{-2}$) | Membrane conductance (S m$^{-2}$) |
|---|---|---|---|
| Cytotrophoblast (amniochorion) | 77 | 18.2 ± 5.0 | 3300 ($\rho = 0.72$) |
| Trophoblast (whole placenta) | 14 | 14.5 ± 7.4 | Assigned zero |
| Retroplacental trophoblast | 14 | 13.1 ± 7 | Assigned zero |

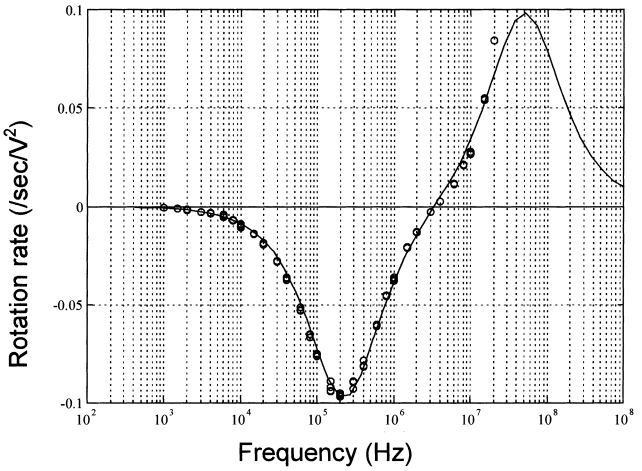


Fig. 3. A ROT spectrum obtained from a typical PMBC at a medium conductivity of 28 mS $m^{-1}$. The data are plotted along with the best fit (solid line) which for this particular cell gave $C_{Mem} = 10.47$ mF $m^{-2}$, $\varepsilon_{cyt} = 55$, $\sigma_{cyt} = 0.33$ S $m^{-1}$ with $\rho = 0.999$

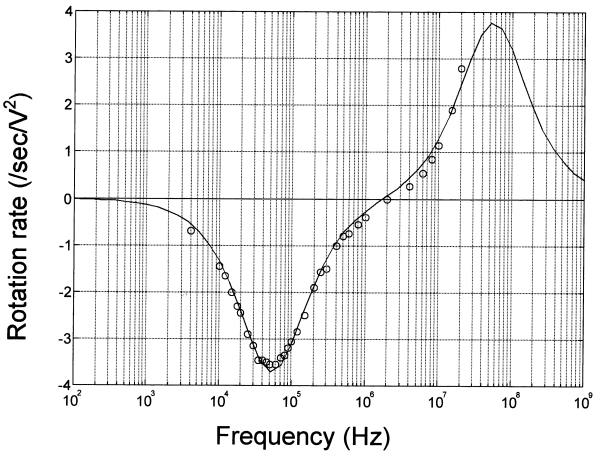


Fig. 4. A ROT spectrum of a trophoblast cell obtained from the amniochorion measured in a medium conductivity of 40 mS $m^{-1}$. The solid line indicates the best fit to the data which for this cell was with: $C_{Mem} = 22.6$ mF $m^{-2}$, $\varepsilon_{cyt} = 60$, $\sigma_{cyt} = 0.33$ S $m^{-1}$, with $\rho = 0.995$.

using Eq. 3 to give a mean specific membrane capacitance of the cells of $13.1 \pm 7$ mF $m^{-2}$.

A summary of the DEP crossover data obtained for the trophoblast cells is shown in Table 2.

### 4.2. ROT

#### 4.2.1. PBMCs

ROT data were obtained for male and female (pregnant and non-pregnant) PBMCs. In general the ROT spectrum was fitted to a single shell model. A typical ROT spectrum for a PBMCs is shown in Fig. 3. The spectrum shown in this figure was obtained for a medium conductivity of 28 mS $m^{-1}$ and reveals an anti-field rotation peak at 200 kHz. The data are plotted along with the best fit (solid line). For this particular cell, the membrane capacitance was calculated to be $C_{Mem} = 10.47$ mF $m^{-2}$ and the other dielectric parameters are shown in the figure legend. 24 cells were analysed by ROT over a range of medium conductivities from 25 mS $m^{-1}$ to 60 mS $m^{-1}$. The average dielectric parameters for all PBMCs (male and female) are shown in Table 3.

#### 4.2.2. Trophoblast cells

*4.2.2.1. Cytotrophoblast from amniochorion.* The ROT spectrum of eight trophoblast cells was measured over a range of medium conductivities from 30 mS $m^{-1}$ to 76 mS $m^{-1}$. Fig. 4 shows a typical spectrum for a trophoblast cell obtained from the amniochorion where the anti-field rotation peak occurs at 50 kHz. This particular spectrum was recorded in a medium conductivity of 40 mS $m^{-1}$ and the best fit to the data is shown by the solid line. For this particular cell the best fit to the data gave a membrane capacitance of $C_{Mem} = 22.6$ mF $m^{-2}$. The dielectric data averaged over all eight cells are shown in Table 3.

*4.2.2.2. Trophoblast from whole placenta.* The

Table 3
Summary of the average values for the specific membrane capacitance for PBMCs and trophoblast cells calculated from ROT data

| Cell type | Number of cells analysed | Membrane capacitance (mF $m^{-2}$) |
|---|---|---|
| PBMCs | 24 | $11.6 \pm 4.2$ |
| Trophoblast (whole placenta) | 38 | $17.8 \pm 9.6$ |
| Cytotrophoblast (amniochorion) | 8 | $26.6 \pm 6.2$ |

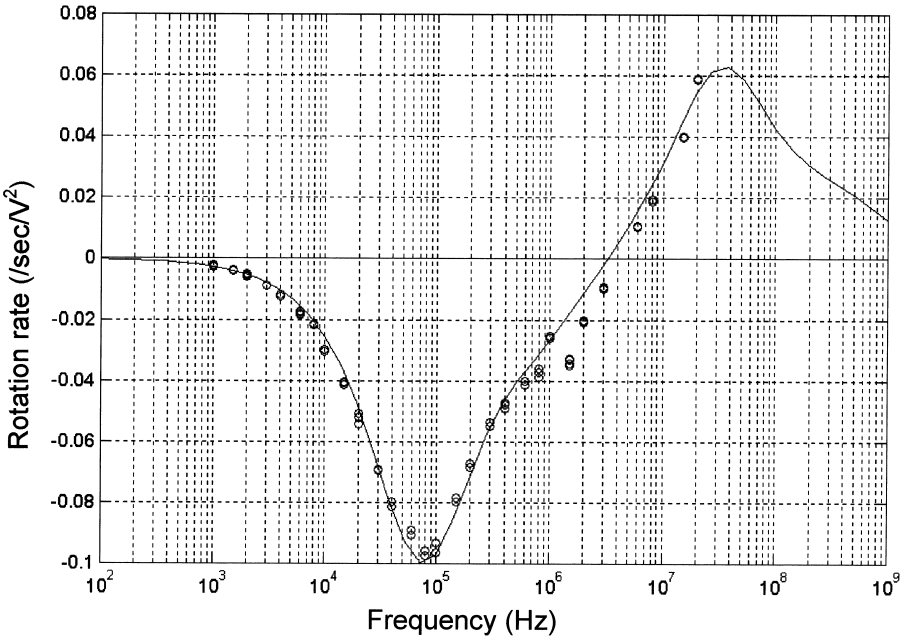


Fig. 5. A ROT spectrum for a trophoblast cell obtained from whole placenta measured in a medium conductivity of 28.6 mS $m^{-1}$. The best fit to the data is shown by the solid line with $C_{Mem} = 13.7$ mF $m^{-2}$, $\varepsilon_{cyt} = 65$, $\sigma_{cyt} = 0.25$ S $m^{-1}$ and $\rho = 0.998$.

ROT spectrum of 38 trophoblast cells was measured over a range of medium conductivities between from 30 mS $m^{-1}$ to 80 mS $m^{-1}$. Fig. 5 shows a typical spectrum for a trophoblast cell with an anti-field rotation peak at 80 kHz. This spectrum was recorded in a medium conductivity of 28.6 mS $m^{-1}$ and the best fit to the data is shown by the solid line. The dielectric data averaged over all 38 cells are shown in Table 3.

## 5. Discussion

### 5.1. Peripheral blood mononuclear cells

The dielectric properties of suspensions of human PBMCs have been measured by a number of research workers with classical dielectric spectroscopic methods [16,17] and by time domain dielectric methods [18]. ROT methods have also been used to measure the dielectric properties of single human lymphocytes [3,19,20]. In the recent work of Yang et al. [20] ROT was used to measure the dielectric properties of T- and B-lymphocytes, monocytes and granulocytes. Their data gave an average membrane capacitance of $10.5 \pm 3.1$ mF $m^{-2}$ for T-lymphocytes and $12.6 \pm 3.5$ mF $m^{-2}$ for B-lymphocytes. These data compare favourably with our measurements of a mixed population of PBMCs. ROT measurements of 24 cells gave an average mean specific membrane capacitance of $11.6 \pm 4.2$ mF $m^{-2}$. Similar values were obtained from DEP crossover measurements of 315 cells, where an average specific membrane capacitance of 11.5 mF $m^{-2}$ was measured.

### 5.2. Trophoblast

During pregnancy large numbers of the placental surface cells break off into the maternal blood, and most of these are trapped and destroyed in the lungs. Therefore, trophoblast cells circulating in the maternal peripheral circulation exist in extremely low numbers and cannot be identified by morphology alone. In order to have a large number of trophoblast cells for study, an appropriate method for preparing cells from the placental tissue is required. To obtain detailed measurements of the dielectric properties of a range of trophoblast cell types, three different methods of cell preparation were used and compared. The choice of methods for the disaggregation of the trophoblast cells was very important, since the dielectric properties of the cells may depend on the prep-

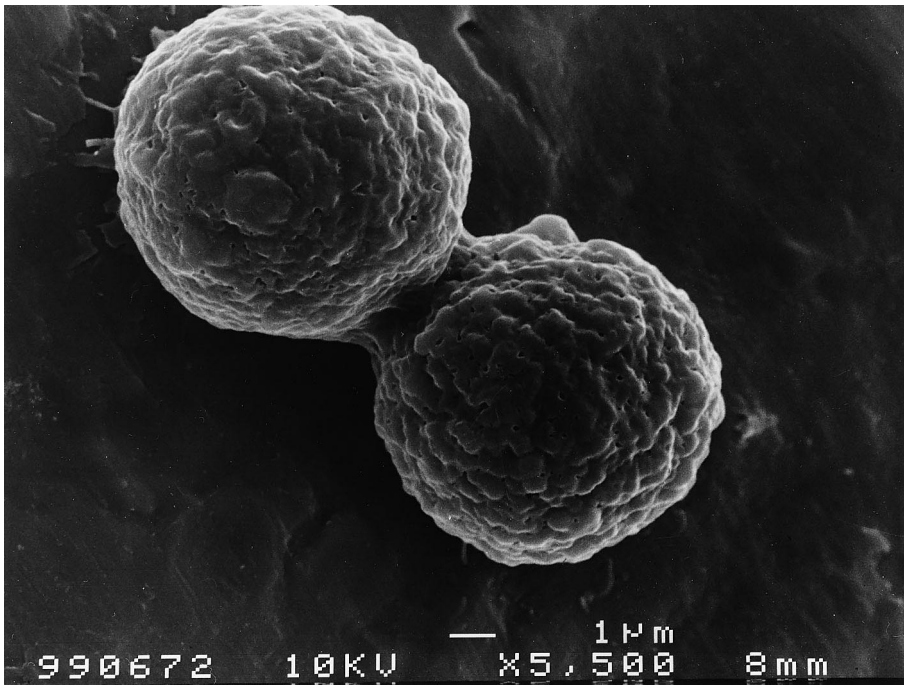


Fig. 6. A scanning electron micrograph of trophoblast cells obtained from amniochorion as described in Section 3. Cells were fixed in a 3% solution of glutaraldehyde in PBS for 1 h, rinsed in PBS and exposed to 1% osmium tetroxide for a further hour. The cells were dehydrated by sequential immersion in 30, 50, 70, 90 and $2\times100\%$ ethanol, followed by a 50:50 ethanol:acetone solution and then 100% acetone followed by sputter coating with gold. Images were taken at a magnification of $5.5\times10^3$ at 10 kV.

aration method. Two general methods of cell preparation were considered *viz* enzymatic disaggregation of the placental tissue and isolation of trophoblast from retroplacental blood. The enzymatic isolation of cells may lead to a loss of membrane proteins and a subsequent change in dielectric properties. Such a preparation will also contain cells that are not trophoblast cells. The preparation of a retroplacental blood sample does not involve enzymatic treatment, however it contains small and variable numbers of trophoblast cells and only the syncytiotrophoblast cells could be used for analysis since cytotrophoblast could not be identified by morphology alone.

Morphological analysis of the cells obtained from whole placental preparations confirmed that the majority were multinucleated and therefore of placental syncytial origin. 99% of the cells were viable whilst approximately 60% of the cells stained positive using the cytoskeletal trophoblast marker JMB2 [13,21]. This is likely to be an underestimate of the percentage of trophoblast cells present since JMB2 may not label all trophoblast cell populations. These percentages are in keeping with the findings of Kliman et al. [12].

Approximately 50% of the cytotrophoblast cells obtained from amniochorion labelled positive with JMB2 as analysed by fluorescent flow cytometry (unpublished data). These data are similar to that reported by Butterworth and Loke [22]. Only cells with intact membranes were selected for analysis by ROT and DEP. Multinuclear trophoblast cells were selected where ever possible to minimise the possibility of PBMCs being analysed.

Retroplacental blood has been shown to contain both syncytiotrophoblast and cytotrophoblast cells, with the ratio of trophoblast cells to lymphocytes varying from 1:22 000 to 1:432 [14]. As detailed in Section 3, the trophoblast cells were easily identified for analysis because they were multinucleated. The importance of this preparation was that it did not involve enzymatic digestion of tissue, thus retroplacental blood could be considered to be source of 'unadulterated' trophoblast cells, and the best model of the in vivo situation.

The membrane capacitance of trophoblast cells

measured by DEP crossover methods is summarised in Table 2 and shows that the capacitance of cells obtained using the three methods of preparation is in all cases higher than that of the PBMCs, see Table 1. The regression coefficient for all PBMCs was close to unity, indicating that the cell population was homogeneous. However, the regression coefficient for the trophoblast cells was 0.72 indicating the heterogeneous nature of these artificially disaggregated cells. The average membrane capacitance of trophoblast cells obtained from retroplacental blood samples was also higher than for PBMCs, but there was a large distribution in the data. This could be accounted for by the heterogeneous nature of the trophoblast population found in blood or by errors in the identification of trophoblast cells by morphology alone.

ROT measurements of trophoblast cells from whole placenta and from amniochorion preparations indicated that the membrane capacitance was similarly increased over that of the PBMC population (Table 3). Again, a wide distribution in the capacitance of cells is apparent for both amniochorion preparations and trophoblast cells isolated from whole placenta, indicative of the heterogeneity of the population.

### 5.3. Comparison of trophoblast and PBMCs and prospects for separation

It has been shown [23] that the membrane capacitance of cells is strongly influenced by the cell membrane area and morphology. The consistent finding of a higher membrane capacitance for trophoblast cells, regardless of the method of preparation, suggests that there is an inherent difference between the trophoblast and PBMCs which does not result from cell preparation techniques. Fig. 7 shows a scanning electron micrograph of two trophoblast cells prepared from the amniochorion. These images show that the surface of the cells is composed of many folds, ruffles and also pits. SEM images of sub-populations of PBMCs were recently published [20] and comparison with these images shows that the trophoblast cells are inherently different. Yang et al. [20] showed that the surface of T- and B-lymphocytes is relatively smooth thus accounting for their relatively low membrane capacitance. Both granulocytes and monocytes have surface projections and ridge like profiles and ruffles leading to an elevated membrane capacitance. It is likely that the degree of folding of the trophoblast membranes as shown in Fig. 6 accounts for the higher membrane capacitance measured for these cells.

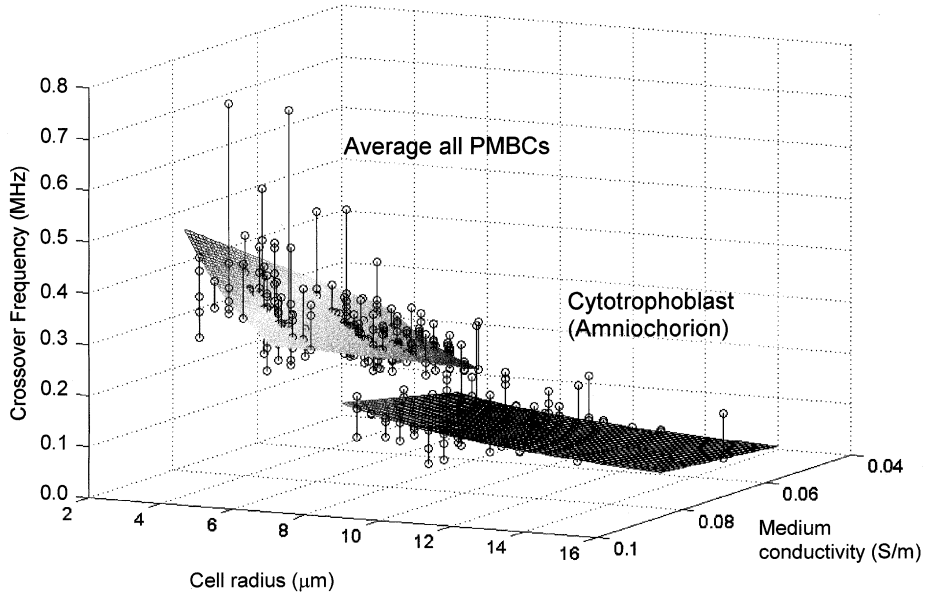


Fig. 7. The DEP crossover frequencies for average PBMCs and for cytotrophoblasts from amniochorion are plotted as a function of medium conductivity and cell radius. The figure shows that DEP separation should be possible at a frequency of around 300 kHz.

DEP cell sorters have been developed by a number of groups [3–7]. These devices operate on the basis that different cells experience different magnitudes and directions of force. Fig. 7 shows the crossover frequencies for trophoblast and PBMCs plotted on the same axis. It can be seen that the average response for the PBMCs and for the cytotrophoblast cells from amniochorion is substantially different, implying that DEP separation would be possible. Thus if DEP separation was performed at a mid frequency of approximately 300 kHz, the trophoblast cells would experience positive DEP whilst simultaneously the PBMCs would experience negative DEP and be repelled from the electrodes. We propose therefore that these differences would lead the two cell types to behave differently in a non-uniform AC electric field, and that this could be used as the basis of a cell separation strategy.

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