# Assessment of the dielectric properties of drug sensitive and resistant leukaemic cells before and after ion channel blockers using dielectrophoresis

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## ABSTRACT

Dielectrophoresis (DEP) is a phenomenon of induced particle motion in non-uniform electric fields. The effect is frequency dependent; by monitoring the motion of particles in AC fields and analysing the change in motion with frequency, it is possible to determine the electrical properties of single cells in lab-on-a-chip systems. In this paper we use DEP to study the electrical properties of drug-sensitive and drug-resistant cancer cells before and after treatment with ion channel blocking agents, which give insight into the origin of cytoplasmic differences shown to have a significant bearing on the origin of drug resistance in cancer.

***Keywords***: ion channel, blockers, conductivity, dielectrophoresis

## 1 INTRODUCTION

Malignant cancers are a major cause of morbidity in western countries. The most common methods of treatment include local excision or radiation and chemotherapy. Chemotherapy is the most effective treatment for metastatic tumours. However, drug resistance is a serious impediment to the successful treatment of many human cancers and is responsible for many deaths each year. One form of classical drug resistance is called multidrug resistance (MDR), characterised by cross-resistance to many anti-cancer drugs that have no common structure. Several mechanisms contribute to the MDR phenotype. A common correlate of MDR is the over-expression of membrane glycoproteins, collectively termed as the ABC (ATP-binding-cassette) transporters. The *MDR1* gene product P-glycoprotein pump (P-gp) has been postulated to cause an increase in drug efflux, which gives rise to reduced and ineffective intracellular drug concentrations. P-gp has variously been reported to be a $Cl^-$ channel [1-3] or bifunctional with one function as a $Cl^-$ channel and another as a peptide transporter [4, 5]. It has also been noted that P-gp shares homology with the cystic fibrosis transmembrane conductance regulator (CFTR) [6, 7], a known $Cl^-$ channel.

Dielectrophoresis is a phenomenon of induced particle motion on the micro- and nano-scale, with a broad range of applications to medicine [8]. If one considers a dielectric particle suspended in a spatially non-uniform electric field, the applied field induces a dipole in the particle; the interaction of the induced dipole with the electric field generates a force. Due to the presence of a field gradient, these forces are not equal and there is a net movement. If the particle is more polarisable than the medium around it, the dipole aligns with the field and the force acts up the field gradient towards the region of highest electric field. If the particle is less polarisable than the medium, the dipole aligns against the field and the particle is repelled from regions of high electric field [9]. The force is dependent on the induced dipole, and is unaffected by the direction of the electric field, responding only to the field gradient. Since the alignment of the field is irrelevant, this force can also be generated in AC fields which has the advantage of reducing any *electrophoretic* force (due to any net particle charge) to zero.

This effect was first termed Dielectrophoresis by Pohl [1978]. The dielectrophoretic force, $F_{DEP}$, acting on a spherical body is given by:

$$F_{DEP} = 2\pi r^3 \varepsilon_m Re\left[K(\omega)\right]\nabla E^2 \tag{1}$$

where *r* is the particle radius, $\varepsilon_m$ is the permittivity of the suspending medium, $\nabla$ is the Del vector operator, *E* is the *rms.* electric field and $Re\left[K(\omega)\right]$ the real part of the Clausius-Mossotti factor, given by:

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \tag{2}$$

where $\varepsilon_m{}^*$ and $\varepsilon_p{}^*$ are the complex permittivities of the medium and particle respectively, and $\varepsilon^* = \varepsilon - \frac{j\sigma}{\omega}$ with $\sigma$ the conductivity, $\varepsilon$ the permittivity and $\omega$ the angular frequency of the applied electric field.

The frequency-dependence of $Re[K(\omega)]$ indicates that the force acting on the particle varies with the frequency. The magnitude of $Re[K(\omega)]$ also varies depending on whether the particle is more or less polarisable than the medium. If $Re[K(\omega)]$ is positive, then particles move to regions of highest field strength (positive dielectrophoresis); the converse is negative dielectrophoresis where particles are repelled from these regions. By careful construction of the electrode geometry which creates the electric field, it is possible to create electric field morphologies so that potential energy minima are bounded by regions of increasing electric field strengths. In such electrodes, particles experiencing positive dielectrophoresis are attracted to the regions of highest electric field (typically the electrode edges, particularly where adjacent electrodes are close), whilst particles experiencing negative dielectrophoresis are trapped in isolated field minima.

Our previous work [10] has shown that drug sensitive (K562) and doxorubicin resistant leukaemic (K562AR) cancer cells exhibited significant differences in their dielectric properties. K562 cells exhibited lower cytoplasmic conductivity- ionic strength (0.23S/m) relative to K562AR (0.50S/m). Broader studies [11] have indicated that there is a strong correlation between drug resistance factor and cytoplasmic conductivity.

In this work, we have DEP to investigate the cellular response of drug-sensitive and drug-resistant leukaemic cells before and after treatment with ion channel blockers in order to determine the contributions of ion channel activity in mediating multi-drug resistance in cancer.

Using DEP, the membrane and cytoplasm of K562 and K562AR cells have now been investigated before and after the use of ion channel blockers (20 μM verapamil, 10 μM NPPB and 0.1 mM quinine). Our results for drug-sensitive cells indicate that there is a significant increase in the cytoplasmic conductivity after blocking the potassium channels with quinine but no corresponding change due to the calcium channel blocker verapamil. However, both verapamil and quinine caused a 25-30% reduction in cytoplasmic conductivity in resistant cells. We suggest that the action of verapamil indicates that calcium channels might be overactive in resistant cells, and would require further investigation. Furthermore, the verapamil results indicated that the cytoplasmic conductivity in resistant cells was reversed to that of the sensitive cells. This finding correlated with earlier findings reporting verapamil as a modulator. The quinine results are less conclusive, however, the results indicate that it is an important channel to explore further, as blocking it caused significant changes to the dielectric properties of both K562 and K562AR. Furthermore, NPPB has shown that blocking the chloride channels in both cell lines resulted in the same $\sigma_{cyto}$ value obtained. This may suggest that P-gp is not behaving like a chloride channel.

# 2 MATERIALS AND METHODS

## 2.1 Chemicals and reagents

Doxorubicin (Sigma Aldrich, Poole, UK) was dissolved in dimethyl sulfoxide (DMSO) [12], and stored frozen as stock solutions, which were thawed prior to use. The same procedure was followed for NPPB, and water was used for quinine and verapamil.

## 2.2 Cell culture and ion channel blockers

Human chronic myelogenous leukaemia (K562), and its doxorubicin resistant counterpart (K562AR), were grown in 20 mM HEPES modified RPMI-1640 medium supplemented with 10% heat-inactivated foetal calf serum (FCS), (Invitrogen, Paisley, UK), 2 mM L- glutamine and 100 units/ mL penicillin-streptomycin. All cell culture reagents were obtained from Sigma Aldrich (Poole, UK), unless stated otherwise. The cells were grown under standard cell culture conditions with 5% $CO_2$ / 95% air at 37°C. K562AR were maintained in the presence of 100nM doxorubicin.

A density of $3\times10^5$ cells per mL were treated with an ion channel blocker, and the cells were incubated for two hours prior to DEP analysis. This procedure was followed using 20 μM (verapamil), 10 μM (NPPB) and 0.1 mM (quinine) at a time.

## 2.3 DEP experiments

The drug resistant and sensitive cells were centrifuged at room temperature at 190 ×g for 5 minutes. The pellets were washed and resuspended in isotonic medium consisting of 8.5% (w/v) sucrose plus 0.3% (w/v) dextrose buffer [13]. The medium conductivity was adjusted to 2.5 mS/m using PBS and the final conductivity, before use, was verified with a conductivity meter (RS components Ltd, London, UK). The final cell population was counted using a haemocytometer and adjusted to approximately $3x10^5$ cells/ mL (±15%) for DEP measurements. In order to reduce the effect of variation in cell number in each sample, the experiments were repeated many times (generally 4-6) with different populations, which were summed prior to modelling.

The dielectric properties of the cells, after each ion channel blocker were determined by fitting the measurement spectra to the single shell model [14]. The best fit model was found by matching the curve to the measured data, and then altering the dielectric parameters of the membrane and the

cytoplasm until a best match was found. For DEP calculations, cell diameters were measured using Photolite software to analyse microscope images of cells.

## 3 RESULTS AND DISCUSSION

The MDR status of K562AR has been confirmed (data not shown) using western immunoblotting and MTT-cytotoxicity testing. The former test confirmed the over expression of P-gp, while cytotoxicity testing showed that K562AR was 14 fold more resistant than its counterpart was.

The collection data of K562 and K562AR before and after treatment with ion channel blockers were obtained (an example of which is shown in Figure 1). The dielectric properties of the membrane and cytoplasm, before and after treatment with ion channel blockers, were estimated using the single shell model to model the average collection data. The results showed significant differences in the cytoplasm of the drug sensitive K562 relative to drug resistant cells (K562AR). As can be seen in table 1, untreated control K562 cells exhibit a lower $\sigma_{cyto}$ (0.3 S/m) relative to the control drug resistant counterpart (0.5 S/m). Treating both cell lines with verapamil (a calcium channel blocker) caused approximately the same degree of reduction in the $\sigma_{cyto}$ (30%). Furthermore, this treatment resulted in the $\sigma_{cyto}$ of K562AR to drop to about the same value as that of the drug sensitive K562.. This result suggests that Verapamil may have reversed the cytoplasmic electrical character of the MDR cell line, K562AR, making it close to that of the drug sensitive K562. This also correlates very well with literature, as Verapamil is a well-known MDR modulator. This agent was one of the first modulators discovered to reverse MDR in cancer patients.

Treating both cell lines with quinine (a potassium channel blocker) resulted in both $\sigma_{cyto}$ values becoming approximately the same (0.45 and 0.40 S/m for K562 and K562AR, respectively). This result may infer a difference in the activity of the potassium channel and that of sodium/potassium pump in the two cell lines, but this requires further investigation. Using NPPB (a chloride channel blocker) resulted in both cell lines exhibiting the same $\sigma_{cyto}$ (0.25 S/m) values. The nature of P-gp has been the source of many contradicting reporting in the literature, some postulating that P-gp is a chloride channel, and others report that it is not. Our DEP result may indicate that P-gp is not a chloride channel, as the blocker would have caused a more dramatic increase in $\sigma_{cyto}$ of K56AR (expressing P-gp), as it would have been blocked. NPPB has resulted in both cell lines exhibiting the same cytoplasmic conductivity.

The results presented here are preliminary in nature and would require some further investigation. However, some interesting and significant differences were found between the drug sensitive and resistant cell lines after treatment with ion channel blockers. Of particular note, is the effect of verapamil in reversing the ionic strength of the cytoplasm of K562AR to that of the drug sensitive K562. Furthermore, NPPB has shown that blocking the chloride channels in both cell lines resulted in the same $\sigma_{cyto}$ value obtained. This may suggest that P-gp is not behaving like a chloride channel.

This paper highlights that DEP is a useful, informative and non-invasive tool for studying the electrophysiological properties of cells before and after drug treatment. Which may have potential for high throughput screening in drug discovery.

| | $\sigma_{cyto}$ (S/m) |
|---|---|
| **K562** | |
| -control | 0.30 (0.28-0.30) |
| -with verapamil | 0.20 (0.20-0.25) |
| -with quinine | 0.45 (0.44-0.48) |
| -with NPPB | 0.25 (0.25-0.27) |
| | |
| **K562AR** | |
| -control | 0.50 (0.48-0.50) |
| -with verapamil | 0.35 (0.32-0.35) |
| -with quinine | 0.40 (0.38-0.40) |
| -with NPPB | 0.25 (0.25-0.27) |

Table 1: A table summarizing the differences in the cytoplasmic conductivities, before and after using ion channel blockers in K562 and K562AR.

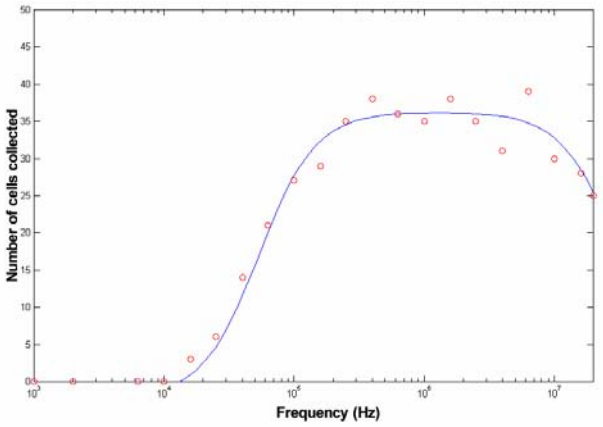


Figure 1: DEP collection data of K562AR after treatment with verapamil

## REFERENCES


[1] MA Valverde, M Diaz, FV Sepulveda et al.“ Volume-regulated chloride channels associated with the human multidrug-resistance P-glycoprotein” Nature, 355 (6363): 830-833, 1992.

[2] GA Altenberg, CG Vanoye, ES Han et al.“ Relationships between rhodamine-123 transport, cell volume, and ion channel function of P-glycoprotein” Journal of Biological Chemistry, 269 (10): 7145-7149, 1994.

[3] RM Wadkins, PD Roepe “Biophysical aspects of P-glycoprotein-mediated multidrug resistance” International Review of Cytology, 171: 121-165, 1997.

[4] DR Gill, SC Hyde, CF Higgins et al. “Separation of drug transport and chloride channel functions of the human multidrug resistance P-glycoprotein” Cell, 71 (1):23-32, 1992.

[5] JT Zhang, V Ling “Involvement of cytoplasmic factors regulating the membrane orientation of P-glycoprotein sequences” Biochemistry, 34 (28): 9159-9165, 1995.

[6] MJ Welsh, MP Anderson, DP Rich et al. “CFTR: a chloride channel with novel regulation” Neuron, 8: 821-829, 1992.

[7] DC Gadsby, AC Nairn “Regulation of CFTR Cl-ion channels by phosphorylation and dephosphorylation” Advances in second messenger and phosphoprotein research, 33: 79-106, 1999.

[8] MP Hughes “Nanoelectromechanics in Engineering and Biology”; CRC Press: Boca Raton, 2002.

[9] R Pethig “Dielectrophoresis: Using inhomogeneous AC electrical fields to separate and manipulate cells” Critical Reviews in Biotechnology , 16 (4): 331-348, 1996.

[10] FH Labeed, HM Coley, H Thomas and MP Hughes “Assessment of multidrug resistance reversal using dielectrophoresis and flow cytometry”, Biophysical Journal, 85: 2028-2034, 2003.

[11] FH Labeed, MP Hughes, HM Coley, “Biophysical characterization of multidrug resistance in MCF-7 cell lines reveals the cytoplasm as a critical factor determining drug sensitivity irrespective of ABC transporter expression” BBA, (2005, submitted).

[12] M Roe, A Folkes, P Ashworth et al. “Reversal of P-glycoprotein- mediated multidtug resistance by a novel anthranilamide dervivatives” Bioorganic & Medicinal Chemistry Letters, 9 (4): 595-600, 1999.

[13] PRC Gascoyne, XB Wang, Y Huang et al. “ Dielectrophoretic separation of cancer cells from blood” IEEE Transactions on industry applications, 33 (3): 670-678, 1997.

[14] A Irimajiri, T Hanai, VA Inouye “Dielectric theory of “multi-stratified shell” model with its application to lymphoma cell” Journal of Theoretical Biology, 78: 251-269, 1979.