Dielectrophoretic assay of bacterial resistance to antibiotics

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Abstract

The dielectrophoretic collection spectra of antibiotic-sensitive and antibiotic-resistant strains of *Staphylococcus epidermidis* have been determined. These indicate that in the absence of antibiotic treatment there is a strong similarity between the dielectric properties of sensitive and resistant strains, and that there is a significant difference between the sensitive strain before and after treatment with the antibiotic streptomycin after 24hrs exposure. This method offers possibilities for the assessment of bacterial resistance to antibiotics.

Keywords

AC electrokinetics; dielectrophoresis; antibiotic resistance; streptomycin; staphylococcus

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1. Introduction

There exists a significant need for a method for rapid assessment of the physical state of bacteria taken from patient samples for the diagnosis and treatment of serious bacterial disease. In order to ensure that treatment is effective, the correct choice of antibiotic must be made as early as possible. This demands procedures which can detect rapidly whether bacteria are affected by a given antibiotic. It is also important to be able to discriminate between dead bacteria (whether killed by an antibiotic or other means), and bacteria that are in a state of dormancy from which they may emerge later causing a relapse or reactivation of the infection.

One family of devices being developed to meet this need are the so-called “laboratories on a chip”: integrated electronic devices with integrated fluid handling, optical analysis and sensing mechanisms with the aim of providing rapid assay of biological samples. A key technology in the development of laboratories on a chip is that of dielectrophoresis, the manipulation of cells and other particles in non-uniform AC electric fields.

Dielectrophoresis has been used for the electrostatic manipulation of cells for over five decades (Pohl 1951); it was first applied to categorisation of the dielectric properties of bacteria in the 1970s in Herbert Pohl’s lab, the results of which were collected in his seminal book in the subject (Pohl 1978). Since then it has been applied as a tool for the detection of bacteria for biotechnology and food applications (e.g. Betts 1994, Suehiro et al. 2003) and separation of bacteria of different species (Markx et al 1996) as well as a method of analysing bacterial properties (e.g. Markx et al 1994, Milner 1998), for the analysis of bacterial motors (Washizu et al. 1993; Hughes and Morgan, 1998) and for the construction of microbial consortia (Alp et al. 2002). Work has been performed on the separation of viable and non-viable bacteria (e.g. Suehiro et al. 2003), though such studies

have rendered bacteria nonviable using methods that cause considerable damage to the cell structure, such as heat treatment.

In this paper, we demonstrate that dielectrophoretic methods can be used to assess the viability of a sample of bacteria both before and after treatment with antibiotics. Using antibiotic-sensitive and antibiotic-resistant strains of *Staphylococcus epidermidis*, we demonstrate that dielectrophoretic methods can detect the changes in both bacteria due to treatment with the antibiotic streptomycin. *S. epidermidis* is a Gram-positive bacterium commonly found on human skin. The results obtained are likely to be applicable also to the closely related but more pathogenic species *Staphylococcus aureus*; both species cause problems in antibiotic treatment due to their variable sensitivity to antibiotics, the problem being most severe with methicillin-resistant *S. aureus* (MRSA) strains.

3. Materials and methods

3.1. Bacteria preparation

Staphylococci are near-perfectly spherical and have a cell wall containing a rigid peptidoglycan matrix surrounding a lipid membrane which encloses a cytoplasm which is non-compartmentalised.. *Staphylococcus epidermidis* strains JD140 (streptomycin sensitive) and JD141 (a streptomycin resistant derivative of JD140), from our own culture collection, were grown in nutrient broth for 16 hours at 37°C, with or without the addition of streptomycin (final concentration 50 mg l⁻¹). After incubation, the bacterial count was determined using a haemocytometer. The bacteria in each culture were harvested by centrifugation and resuspended in 280mM mannitol solution with phosphate-buffered saline (PBS) immediately prior to experimentation. Complete sets of experimental data were obtained for concentrations of PBS corresponding to medium conductivities of

1mSm⁻¹ and 2mSm⁻¹ in order to introduce additional variables to enhance the modelling of data. After resuspension, the bacteria were counted again using the haemocytometer and adjusted to a cell concentration of 10⁵ ml⁻¹ and a total volume of 7ml. Plate counts of viable (colony-forming) bacteria confirmed that streptomycin killed JD140 effectively, with no colonies recovered after 6 hours of treatment (survival <0.3%), while JD141 remained fully viable (and showed an increased colony count).

3.2. *Dielectrophoresis setup*

Electrodes consisting of sharp syringe needles were glued to the outer rim of a glass Petri dish; the needles were bent to an appropriate angle for the flat edges of the needles to lie flat across the bottom of the Petri dish. This ensured that the edges of the needles were all in the focal plane of the microscope and that no cells could collect under the needles. The distance between opposing needle tips was measured at 130µm, and the tips of the needles pointed directly towards one another. Wires were soldered to the needles at sufficient distance to ensure the solder did not come into contact with the cell suspension once it was placed in the Petri dish. This setup is described in further detail by Labeed *et al.* (2003). The electrodes were energised by a Thurlby-Thandar signal generator providing a 20V_{pk-pk} signal in the range 1Hz-20MHz. The experiments were observed using an Olympus inverted microscope, with x400 total magnification.

4. Results and discussion

A 7ml sample of cells was inserted into the Petri dish. The numbers of particles collected at the electrode edge were counted at a range of frequencies between 100kHz and 16MHz, the lower limit being chosen to avoid effects due to electro-osmotic fluid flow (Ramos *et al.* 1999) this procedure was repeated at five frequencies per decade. The collection

spectra are shown in figure 1. As can be seen, the collection spectra of untreated JD140 (figure 1a) and JD141 (figure 1b) bacteria exhibit a monotonically decreasing profile with frequency. This is also observed in JD141 after 24 hours exposure to streptomycin (figure 1d). However, after 24 hours exposed to streptomycin, the JD140 sample exhibited a significantly different profile, including a doubling of collection numbers between 100kHz and 1MHz (figure 1c). When the sample was exposed for two days or longer, the collection at frequencies below 200kHz stopped completely (figure 1e).

In order to understand these processes, the dielectric properties of the particles can be estimated using the multi-shell model developed by Irimajiri et al. (1979). The dielectric properties were determined using the smeared-out-shell model with three shells representing the cytoplasm, cell membrane and cell wall. A multiplier factor was used in order to scale the results to fit the Clausius-Mossotti factor described in equation (2) to the number of bacteria collected. The fit was performed by visual inspection, based initially on known parameters such as the dimensions of the various layers of the bacteria and estimated parameters based on those found in the literature (e.g. Hughes and Morgan 1999). The parameters were then changed until the line successfully matched to the collection profile of all the experimental data for a particular cell type and condition. The estimated parameters determined by the model are displayed in table 1. The parameters were then varied from this best case until the line passed through the extreme limits of the data set in order to find the range of values which that parameter could take. This procedure is described in more detail by Hughes *et al.* (2002)

The model was in some cases found to be insensitive to the value of permittivity of either the cell wall or the cytoplasm, with values between $5\epsilon_0$ and $500\epsilon_0$ producing acceptable

results. The dimensions used in the model are as follows: bacterial radius $0.5\mu\text{m}$, total plasma membrane thickness 10nm , and wall thickness 25nm , in accordance with published descriptions of the fine structure of staphylococci (e.g. Cohen 1972). The value of plasma membrane thickness is often described as 5nm from leaflet centre to leaflet centre, but in this model, we are considering the whole thickness of the structure.

Prior to treatment with streptomycin, both strains of bacteria have very similar collection spectra. The cell wall conductivities of the sensitive and resistant strains were estimated at $300\pm 50\text{mSm}^{-1}$ and $275\pm 25\text{mSm}^{-1}$ respectively. Whilst the model for JD140 proved insensitive to changes in cell wall permittivity, the model for JD141 was more sensitive and produced an estimate of $75\epsilon_0\pm 25$. This compares well with data derived by Carstensen and Marquis for isolated *Micrococcus lysodeikticus* cell walls in low-conductivity media (Carstensen and Marquis 1968), who reported conductivities of approximately 300mSm^{-1} in low conductivity media. The membrane permittivities were found to be $4\text{--}5\epsilon_0$, in agreement with a large body of experimental data for cell membranes (e.g. Gascoyne *et al.* 1993). Membrane conductivity was estimated to be less than 0.1mSm^{-1} , and sufficiently low to be considered negligible. Whilst the model was insensitive to cytoplasmic permittivity, the cytoplasmic conductivities were estimated to be similar at $200\text{mSm}^{-1}\pm 50$.

Where the untreated cells showed similar properties, substantial differences were observed in the bacteria that had been treated with streptomycin. Considering the resistant strain first, it might be expected to be similar to the untreated bacteria. Compared to the model for untreated JD141 bacteria, the model was more sensitive to changes in permittivity, showing values of $50\epsilon_0$ for the cytoplasm and value less than $100\epsilon_0$ for the cell wall (the

model not changing for values below this threshold). The model was also more sensitive to conductivity variation, with cytoplasmic conductivity having an estimated value of 150mSm^{-1} accurate to within 10%.

After one day's exposure to streptomycin (figure 1d), the sensitive bacteria exhibited a collection profile to which no model could be found to fit. However, a good fit could be found to the collection profile after two days. Furthermore, an accurate fit to the collection profile data could be found by combining the spectra of untreated and 2-day-exposed bacteria, suggesting that the sample is composed of a heterogeneous population of bacteria which have been affected by the antibiotic and those that have not, and that after 2 days the bacteria have all been affected.

Considering the 2-day exposure (Figure 1e), the model suggests that the cell wall permittivity has values greater than $50\epsilon_0$, but that properties were similar to the untreated bacteria, with the exception of the cell wall. According to the modelled results, the cell wall conductivity dropped by over an order of magnitude to approximately 10mSm^{-1} after two days exposure for the same wall thickness; a similar result could be achieved for higher values of conductivity but correspondingly lower values of cell wall thickness, indicating a general reduction in the net charge within the wall. As such, this effective change in charge could be attributed either to a reduction in the thickness of the cell wall or to the disintegration of the wall structure, leaving less charged material present. Streptomycin is known to interfere with RNA transcription rather than acting on the cell wall directly, the disintegration of the cell wall may be attributable to the general breakdown of cellular processes due to the action of the antibiotic.

This result is significant since it demonstrates a straightforward method for assaying the viability of bacteria and the effect of antibiotics on bacteria. By applying an electric field of frequency 100-200kHz, the bacteria will either be observed moving to regions of high electric field (indicating viability) or low electric field (indicating nonviability). Since this test does not depend on bacterial growth or colony-forming ability, it may have potential for investigating slowly-reproducing bacteria such as *Mycobacterium tuberculosis*.

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Figure 1. Collection spectra (illustrated by circles) for drug-sensitive (JD140) and drug-resistant (JD141) bacteria with best-fit lines according to the model described in the text. A. Sensitive bacteria; B. Resistant bacteria; C. Sensitive bacteria, one day after treatment with streptomycin; D. Resistant bacteria two days after treatment with streptomycin. E. Sensitive bacteria, two days after treatment with streptomycin.

	Without streptomycin		With streptomycin	
	JD140	JD141	JD140	JD141
$\sigma_{\text{wall}} (\text{mSm}^{-1})$	300±50	275±25	10±2	250±50
$\epsilon_{\text{wall}} (\epsilon_0)$	Insensitive	75±25	>50	<100
$\sigma_{\text{membrane}} (\text{mSm}^{-1})$	Negligible	Negligible	Negligible	Negligible
$\epsilon_{\text{membrane}} (\epsilon_0)$	4±.5	4.5±.5	4±.5	4±.5
$\sigma_{\text{cytoplasm}} (\text{mSm}^{-1})$	200±50	200±50	200±50	150
$\epsilon_{\text{cytoplasm}} (\epsilon_0)$	Insensitive	Insensitive	insensitive	50±25

Table 1. The parameters used to generate the best-fit lines shown in figure 1. The data show the dielectric parameters for sensitive (JD140) and resistant (JD141) bacteria without and with treatment by streptomycin. Error margins are shown where the margin of error is greater than $\pm 10\%$. In all cases, the radius of the cytoplasm was 500nm, the thickness of the membrane 10nm, and the thickness of the cell wall, 25nm.