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**Rapid determination of bacterial susceptibility to antibiotics combining Raman spectroscopy and Dielectrophoresis**

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**Introduction**

The development of rapid, sensitive and specific methods to determine antibiotic susceptibility of bacteria is required to help reduce the widespread misuse of antibiotics and the growing multidrug-resistance problem [1]. This study presents a combined Raman spectroscopic and dielectrophoretic (DEP) approach to obtain direct, real-time measurements of a suspension of planktonic bacteria without the need of any labelling or other time-consuming sample preparation processes. Thanks to the spatial non-uniform DEP fields, bacteria are easily captured and concentrated under the laser spot of the Raman apparatus, increasing the sensitivity of the Raman technique [2]. Optimizing the setup conditions we were able to characterize different bacterial strains with high specificity. Using our Raman-DEP device, we demonstrated the susceptibility of *E. coli* towards the commonly prescribed second-generation fluoroquinolone (3) ciprofloxacin (CP), after only one hour of treatment, by monitoring spectral changes in the chemical fingerprint of the bacteria, which are related to the mode of action of the drug. Comparison between treated and untreated samples were performed at the MIC (minimum inhibitory concentration) and sub-MIC levels for different time points over a 3 hour span, and the Raman data were processed by supervised multivariate tools, such as PLS-Discriminant Analysis and PLS-Regression, for the calibration of descriptive models of cellular modifications. The models were validated using the cross-validation strategy. Simultaneously, standard microbiological assays based on cell viability, turbidity test and fluorescence microscopy, were carried out as reference methods to correlate the observed Raman response and to build strong predictive models.

**The DEP device for Raman analysis of bacteria**

Dielectrophoresis (DEP)

- Dielectric particles in a non-uniform electric field causing force
- Magnitude of the force depends on permittivity of both particle and medium
- Can be positive or negative depending on which electrical permittivity is higher

A dielectric particle placed in an electric field becomes electrically polarized as a result of partial charge separation, which leads to an induced dipole moment. The dipole moment is a consequence of the generation of equal and opposite charges at the boundary of the particle. In a non-uniform electric field, the particle experiences a net dielectrophoretic force. The magnitude of the induced dipole depends on the polarizability of the particle with respect to that of the medium. Applying the correct voltage and frequency, dielectric particles, such as bacteria, can be manipulated into clusters to increase their local concentration.

**Raman-DEP device**

**Raman spectra of Bacteria**

**Dielectrophoresis (DEP)**

**Raman-DEP device**

**Raman spectra of Bacteria**

**E. coli susceptibility to ciprofloxacin measured by Raman-DEP**

**Calibration of the susceptibility test method**

**Experimental setup:** Bacteria were treated with 1 µg/ml of ciprofloxacin in the middle of their exponential growth phase, when their OD_{600} was 0.3, during which they are more sensitive to the antibiotic.

**Fluorescence Microscopy**

- Raman spectra of CTRL and treated bacteria collected over time with the DEP device
- Principal Components Analysis (PCA) helps in the visualisation of non random variation in spectral data of CTRL and Treated bacteria. The spectra of E. coli treated with CP are grouped in the PCA scores plot.
- PCA also revealed that Raman spectra captured changes related to the passage of time in both CTRL and Treated samples.
- This effect was taken into consideration for subsequent classification analysis.

**Conclusions**

The Raman-DEP device here described allows to characterize different bacterial strains with high specificity and to follow dynamic interactions of the bacteria with antibiotics.

Raman data processed with supervised multivariate data analysis are able to detect subtle spectral differences at a molecular level between treated or untreated bacterial cells after only 1 hour of treatment.

This Raman-DEP method could open the way to rapid bacterial antibiotic susceptibility test without the necessity of time consuming sample preparation and overnight incubation required by classical microbiological techniques.

**References**