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Raman Microspectroscopic Studies on Differentiating Bacteria, Detecting Antimicrobial Resistance and Delineating Biomarkers of Sepsis

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ABSTRACT

Over the last few decades, the development of several new techniques as well as sophisticated instruments have contributed to a better understanding of biological systems. Among these, Raman spectroscopy has emerged as an indispensable tool. Traditionally a chemist's tool, Raman spectroscopy has recently found numerous applications in the field of biology and medicine. Of particular advantage is the fact that Raman spectroscopy is non-invasive, non-destructive, label- free, requires minimal sample volume and offers multi-component analysis in a single scan. Biological samples are complex and are made up of several biomolecules like proteins, lipids, carbohydrates and nucleic acids. These molecules have unique structures and, therefore, yield unique spectral fingerprints. The structural changes in the biomolecules can be tracked during disease or any other biological process. In short, a Raman spectrum reflects a biological entity's underlying chemistry and any perturbation in the cellular chemistry can be tracked efficiently and rapidly. However, interpreting Raman spectra obtained from complex biological systems like cells, tissues and body fluids can be challenging. Therefore, multivariate statistical algorithms like principal component analysis and discriminant analysis have to be employed to enable the extraction of useful information. In the present work, multiple applications of Raman spectroscopy were demonstrated: identification of two closely related bacterial strains, tracking the emergence of antimicrobial resistance in bacteria and delineating biomarkers of sepsis in mice model systems as well as human patient samples.

In the first part, the utility of Raman spectroscopy in differentiating two very closely related strains of Mycobacterium was demonstrated. The strains Mycobacterium indicus pranii (MIP) and Mycobacterium intracellulare cannot be differentiated using conventional methods such as 16s rRNA sequencing owing to their identical sequences. MIP is known for its immuno-modulatory properties and shows adjuvant-like properties against leprosy, tuberculosis and cancer. M. intracellulare, on the other hand, is an opportunistic pathogen and causes severe lung infections, especially in AIDS patients. Thus, distinguishing these two strains is of clinical relevance since they may often co-exist in immuno-compromised individuals. A combinatorial approach of Raman spectroscopy and multivariate data analysis revealed that MIP could be differentiated from M. intracellulare on the basis of mycolic acids and carotenoids.

In the second part, the development of Raman spectroscopy as a surveillance technology for rapid detection of antimicrobial resistance in bacteria was shown. Antimicrobial resistance has become a major health care concern and is declared as one of the top ten global threats by the World Health Organisation. Traditional methods for detecting antimicrobial resistance are mostly growth-based assays which take at least 24-48 hours. Therefore, the development of technologies that can rapidly detect antimicrobial resistance has become critically important. Using a series of genetic mutants of Escherichia coli that display differential susceptibilities towards antibiotics, the potential of Raman spectroscopy in rapidly detecting the biomolecular changes occurring as a result of ciprofloxacin treatment was demonstrated. In addition, it was also shown that Raman spectroscopy could detect the emergence of antimicrobial resistance in a sensitive strain within 6 hours.

In the third part, the efficacy of Raman spectroscopy in detecting biomolecular changes during sepsis was explored in both mice model systems and human patient samples. Sepsis is a leading cause of mortality in the intensive care units (ICUs) of hospitals worldwide and in its early stages, is difficult to diagnose, as many of its symptoms mimic other medical conditions. The timely diagnosis of sepsis is the key to improve prognosis. Current methods for diagnosis include blood culture, measuring serum cytokines, lactate and procalcitonin levels as well as organ- function assessments using the SOFA score. Most of these methods are non-specific and time-consuming. The search for a diagnostic tool that can rapidly diagnose sepsis has still been unsuccessful. Here, the potential of Raman spectroscopy in rapidly identifying biomarkers of sepsis in a mouse model was evaluated. A sepsis-like condition was induced in different strains of mice using Salmonella Typhimurium. The modulated Raman markers were indicative of extensive hemolysis occurring as a result of sepsis. Raman spectroscopy could also detect a higher degree of hemolysis when sepsis was induced in the Nos2-/- immune-compromised strain of mice. The application was extended to human sepsis patients and the potential of this technique in differentiating sepsis and shock from healthy controls was evaluated. To determine the specificity of Raman markers towards sepsis, other inflammatory conditions like Systemic Lupus Erythematosus (autoimmune disorder) and Dengue (viral infection) was also investigated. It was shown that some of the identified Raman markers were sepsis-specific and were not present in other inflammatory conditions.

Overall, this study demonstrates the potential of Raman spectroscopy as a novel biophotonics- based method for identification of bacteria, scoring antimicrobial resistance and identifying potential biomarkers for sepsis in mice models and human patients.