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Tuberculosis determination using surface enhanced Raman spectroscopy and Chemometric methods

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Abstract

Recently applications of Raman spectroscopy have been increased to various fields, especially clinical diagnosis. Tuberculosis (TB) is one of the leading causes of mortality in the world. WHO estimated that 10.4 M people were detected as active TB and about 1.4 M people died in 2015. However, propagation of TB can be prevented by early diagnosis and treatments. Various methods have been used for diagnosis TB such as sputum smear microscopy (SSM), culture method, chest radiography, tuberculin skin testing, etc. The existing methods are poorly sensitive and time-consuming. So, there is a need to develop a rapid and sensitive method to detect TB. Surface enhanced Raman spectroscopy (SERS) is an alternative nondestructive method in terms of its rapid and sensitivity. Herein, two groups of serum protein samples, active TB (AT) and healthy control (HC) were selected for SERS analysis and spectra were measured with 785 nm laser. The measured spectra associated with vibrational modes of serum proteins. To analyze the data various multivariate statistical methods such as principal component analysis (PCA), support vector machine (SVM), decision tree (DT) and random forest (RF) were developed and tested their ability to discriminate the HC and AT samples. First two principal components (PC1, PC2) PCA scores showed clusters of AT and HC separately. A blind test has been done to validate the calibration model and test data currently falls under the same category. Results demonstrate the distinction between HC and AT samples.

Tuberculosis (TB) is the ninth leading cause of death worldwide and the leading cause from a single infectious agent, based on the WHO Global Tuberculosis Report in 2017. TB causes massive health care burdens in many parts of the world, specifically in the resource constrained developing world. Most deaths from TB could be prevented with cost effective early diagnosis and appropriate treatment.

Conventional TB detection methods are either too slow as it takes a few weeks for diagnosis or they lack the specificity and accuracy. Thus the objective of this study was to develop a fast and efficient detection for TB using surface enhanced Raman scattering (SERS) technique.

SERS spectra for different forms of mycolic acids (MAs) that are both synthetic origin and actual extracts from the mycobacteria species were obtained by label-free direct detection mode. Similarly, we collected SERS spectra for γ-irradiated whole bacteria (WB). Measurements were done using silver (Ag) coated silicon nanopillar (Ag SNP) as SERS substrate.

We report the SERS based detection of MA, which is a biomarker for mycobacteria species including Mycobacterium tuberculosis. For the first time, we also establish the SERS spectral characterization of the three major forms of MA – αMA, methoxy-MA, and keto-MA, in bacterial extracts and also in γ-irradiated WB. We validated our findings by mass spectrometry. SERS detection of these three forms of MA could be useful in differentiating pathogenic and nonpathogenic Mycobacterium spp.

We have demonstrated the direct detection of three major forms of MA – αMA, methoxy-MA, and keto-MA, in two different types of MA extracts from MTB bacteria, namely delipidated MA and undelipidated MA and finally in γ-irradiated WB. In the near future, this study could pave the way for a fast and efficient detection method for TB, which is of high clinical significance.

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