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Title: Surface-enhanced Raman spectroscopy for rapid identification of common pathogenic bacteria: a pilot study

Introduction: Rapid identification of disease-causing bacterial pathogens is crucial for the appropriate initiation of antibiotic treatment. Raman spectroscopy promises culture-free and rapid bacterial detection and identification. In this pilot study, we used surface-enhanced Raman spectroscopy (SERS) to generate a spectral dataset of 6 common bacterial pathogens and applied multivariate analysis to identify each pathogen.

Methods: *A. baumannii (ATCC 19606)*, *E. coli (ATCC 25922)*, *P. aeruginosa (ATCC 27853)*, *S. aureus (ATCC 29213)*, *S. pneumoniae (ATCC 49619)*, and *K. pneumonia (NCTC 13440) were* obtained from NordLab, Oulu, Finland. Each bacterial pathogen (10µl) was collected from a single colony and placed on stainless steel mirror plates. The bacterial biomass was made inactive by placing them at 37ºC for 20 minutes. Subsequently, a small drop (1-2µl) of reactant-free gold nanoparticles (AuNPs; diameter = 150nm, stabilized suspension in 0.1MM PBS, Sigma-Aldrich) was deposited onto the bacterial biomass and allowed to dry at room temperature. The drop was examined by a light microscope and Raman scattering was collected from the droplet's edges. A Thermo Scientific™ DXR™2xi confocal Raman Imaging Microscope equipped with a 100x/0.8 air objective and full range grating (50-3250 cm^{-1}; spectral resolution = 5 cm^{-1}) was used to collect SERS spectra. To excite the Raman signal, a 785 nm laser (25 mW) and a 50 µm confocal pinhole aperture were used and spectra were collected for 1s. From each bacteria, 1000 spectra were collected from 5 different locations (200 spectra/location). The raw spectra were truncated to the fingerprint region (350-1750 cm^{-1}), noise-minimized (principal component analysis), baseline-corrected (asymmetric least squares), and vector normalized. Finally, the pre-processed spectra were divided into two sets (70% training and 30% testing) and fed to a partial least squares-discriminant analysis (PLS-DA) model (9 latent variables) for discriminating into six classes, i.e., six bacteria pathogens.

Results: Even with the relatively low reproducibility of AuNPs, the PLS-DA model was able to detect different bacteria pathogens with high overall accuracy (97%). Sensitivity values are 95%, 98%, 99%, 91%, 96%, 100% and specificity values are 100%,100%, 98%, 99%, 99%, and 100% for *A. baumannii*, *E. coli*, *P. aeruginosa*, *S. aureus*, *S. pneumoniae*, and *K. pneumonia,* respectively.

Discussion: Our SERS approach with commercially available AuNPs shows potential for culture-free and rapid bacterial detection. This provides a baseline for the methodological development using ordered plasmonic nanostructures for more reproducible SERS signals and could be eventually extended for clinical diagnostics using biofluids.