SERS detection of pneumonia in breath of children with cystic fibrosis

Cystic fibrosis (CF) is the most frequently inherited disease in the Western world, and also the one with the highest morbidity and mortality. The main reason is chronic lung infections caused by the pathogenic bacterium Pseudomonas aeruginosa, which is well-adapted to the thick and dehydrated mucous in the CF airways. Established methods to detect P. aeruginosa in young CF children are invasive and lack sensitivity, which is why novel approaches are being investigated. P. aeruginosa emits hydrogen cyanide (HCN) gas, which can possibly be used as a biomarker for early P. aeruginosa colonisation, if it can be detected in the breath. It was investigated if a nanopillar substrate for surface-enhanced Raman spectroscopy (SERS), developed in the Nanoprobes group, could be optimised for gas phase detection of HCN. The project consisted of 3 steps, of which the first was to establish a chemical method to detect cyanide on the substrate in relevant concentrations, preferably in gas. Step I was split up into two parts; one for HCN detection in the gas phase, and one for detection of potassium cyanide (KCN) in serial dilutions to reach sufficiently low CN concentrations and verify the limit of detection. Once this was done, Step II was to measure HCN(g) from emissions of P. aeruginosa; first from the established reference strain, the wild type PAO1. Secondly, it was relevant to study clinical P. aeruginosa strains, isolated for the first time from CF children (the wild type-like strains), and then compare to SERS measurements on later strains, isolated from the same patients after their infection became chronic and the P. aeruginosa had mutated in the lasR gene, which is essential to HCN production. Step III was a clinical trial, where children with CF would blow into a bag containing the SERS substrate, which was then measured, to see if HCN was detected when a new P. aeruginosa colonisation occurred; and data was correlated to culturing of sputum from the patient's lungs.

The SERS substrate was optimised, and setups were developed for HCN(g) detection, for SERS detection of HCN from bacterial volatiles, and for collection and SERS substrate exposure to human breath. Five ppm HCN was successfully detected in gas phase, and KCN was detected down to 10^-6 M. HCN detection was demonstrated from cultures of P. aeruginosa wild types, starting from the end of exponential / beginning of stationary growth phase. HCN was also detected from lasR mutated clinical P. aeruginosa strains isolated from the airways of children with CF, when the mutation was located at the 5' terminal (downstream) of the gene. P. aeruginosa isolates with a mutation at the 3' terminal of the lasR gene (upstream) did not emit detectable HCN. Application for ethics' committee was submitted and permission granted to conduct a 4 months' clinical pilot study at Rigshospitalet, including 50 CF patients aged 5-17 years and 19 age-matched control subjects. One CF patient had a new P. aeruginosa lung colonisation during the trial, and it was probably detected on the SERS substrate, which had an increased HCN signal compared to the patient's other visits. Additional cases of increased SERS cyanide signal were seen in the breath of some of the children, and it was speculated if they could come from prolonged exposure time or from children being exposed to passive smoking at home. The SERS substrate has a background peak in the Raman spectrum, which needs to be addressed, because it is located very close to the cyanide peak.