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Review

Raman spectroscopy towards clinical application: drug monitoring and pathogen identification

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ABSTRACT

Raman spectroscopy is a label-free method that measures quickly and contactlessly, providing detailed information from the sample, and has proved to be an ideal tool for medical and life science research. In this review, recent advances of the technique towards drug monitoring and pathogen identification by the Jena Research Groups are reviewed. Surface-enhanced Raman spectroscopy (SERS) and ultraviolet resonance Raman spectroscopy in hollow-core optical fibres enable the detection of drugs at low concentrations as shown for the metabolites of the immunosuppressive drug 6-mercaptopurine as well as antimalarial agents. Furthermore, Raman spectroscopy can be used to characterise pathogenic bacteria in infectious diseases directly from body fluids, making time-consuming cultivation processes dispensable. Using the example of urinary tract infection, it is shown how bacteria can be identified from patients' urine samples within <1 h. The methods cover both single-cell analysis and dielectrophoretic capturing of bacteria in suspension. The latter method could also be used for fast (<3.5 h) identification of antibiotic resistance as shown exemplarily for vancomycin-resistant enterococci.

1. Introduction

Raman spectroscopy has proved to be an ideal tool for medical and life science research, as Raman measures without contact, providing label-free information on processes within living cells without disturbing them. Furthermore, Raman spectroscopy measures quickly, overcoming the need for complex and time-consuming laboratory analyses in many cases. Raman spectroscopy also measures precisely, providing the ultrasensitive detection capabilities needed for clinical applications. Last but not least, Raman spectroscopy can easily be combined with other optical and non-optical methods to enable convenient sample handling and processing of clinical patient samples. In combination with a microscope, high spatial resolution (<1 μm) can be achieved, enabling the analysis of single bacterial cells. As water yields only a very weak Raman spectrum, it is the ideal solvent for Raman spectroscopic analysis. Thus, analysis of body fluids can be carried out by means of Raman spectroscopy [1–3].

In the following, the results presented at the 6th European Conference on Bloodstream Infections, on 6–7 June 2015 in Vravrona, Greece, will be summarised. They cover recent research highlights from the Jena Research Group dealing with Raman spectroscopy-based concepts for the detection of drugs and the technological developments towards therapeutic drug monitoring, the detection and identification of bacteria from body fluids with a special focus on urine from patients suffering from urinary tract infections (UTIs), as well as spectroscopic approaches for fast antibiotic susceptibility testing.

2. Therapeutic drug monitoring

For the detection of low concentrations of drugs, enhancement methods need to be applied. Here especially, surface-enhanced Raman spectroscopy (SERS) enables monitoring of substances at low concentrations [4]. Combining SERS with a lab-on-a-chip (LOC) microfluidic device enables enhancement of the reproducibility of the analysis [5,6]. By means of LOC-SERS it is possible to detect and quantify antibiotics at micromolar concentrations, which are in the therapeutically important range [7,8]. An alternative approach to detect analytes that cannot be directly monitored by means of LOC-SERS is an indirect detection of a fluorescence dye that can react with the analyte [9].

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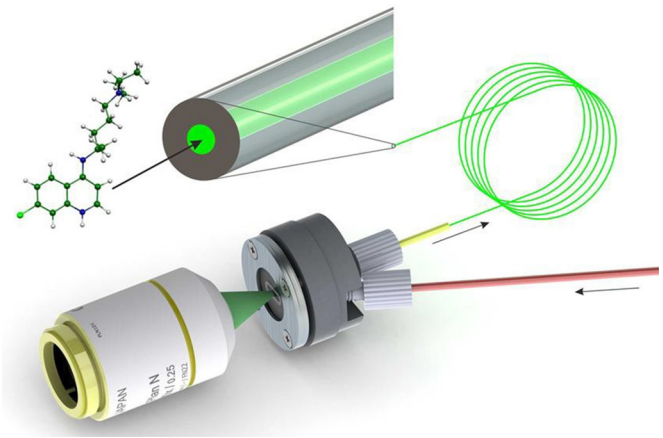

Fig. 1. Schematics of the fibre sensing setup. The analyte is injected into the fibre with a syringe pump. Laser light is coupled into the same fibre to interact with the analyte over a large distance (fibre length) enabling the detection of low concentrations. The backscattered Raman signal is collected through the same objective lens and is further analysed in a spectrometer with a charge-coupled device (CCD) camera.

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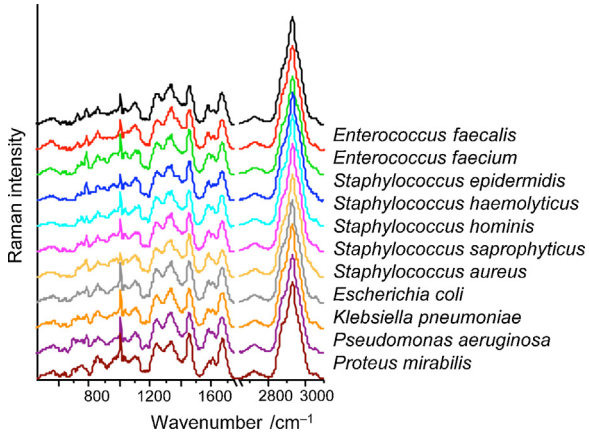

Fig. 2. Mean Raman spectra of major pathogens in urinary tract infections used to construct a support vector machine classification model for the identification of unknown patient samples.

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LOC-SERS is not only suitable for identifying and quantifying pharmacologically active substances. It can also be used to monitor the therapeutic efficacy of drugs with respect to enzyme activity, which may inactivate the drug too fast or not at all. An example is thiopurine methyltransferase (TPMT) activity in red blood cells. Since the concentration of toxic and active metabolites of the immunosuppressive drug 6-mercaptopurine is rather high in patients, therapy using this drug can result in serious toxicity as well as failure of efficacy owing to genetic differences in metabolising enzymes. A huge variety of TPMT genotypes exhibiting different activities for the methylation of thiopurines impede determination of a therapeutic dosage, since high enzyme activity results in the inactivation of thiopurines, whereas low activity can lead to toxic effects. Here, LOC-SERS was used successfully to determine the TPMT activity in blood samples [10].

An alternative method to detect therapeutic agents is fibre-enhanced Raman spectroscopy (FERS). Here, a hollow-core optical fibre is used to enhance the Raman signal significantly in order to detect even lower concentrations. Application of an ultraviolet excitation wavelength in combination with a hollow-core optical fibre can even detect chloroquine and mefloquine at concentrations <100 μM in an aqueous environment (Fig. 1) [11].

Applying visible excitation wavelengths enables direct monitoring of human breath, which is a mixture of different major compounds including N_2, O_2, CO_2 and H_2O as well as traces of volatile organic compounds. In addition, there are important gaseous markers for the detection of different diseases such as, e.g., acetone (C_3H_6O) and methane (CH_4) for lung cancer, or NH_3 and $^{12}CO_2$ for *Helicobacter pylori* infection. Application of FERS with a hollow-core optical fibre allows the detection of all sorts of gaseous components in human breath. Even the differentiation between isotope-labelled substances is possible in routine measurements [12].

Applying a microstructured hollow-core photonic crystal fibre for FERS even allows simultaneous monitoring of H_2 in the presence of all other gases such as, e.g., CH_4, N_2, O_2 and CO_2. This was achieved by a combination of rotational Raman spectroscopy for H_2 and vibrational Raman spectroscopy for the other gases. With this approach, it was possible to detect H_2 down to a limit of detection of 4.7 ppm besides other gases (CH_4, N_2, O_2, $^{12}CO_2$ and $^{13}CO_2$) [13].

3. Diagnosis of infectious diseases from body fluids

The prevalent causes of death in non-cardiology intensive care units are pathogen-induced sepsis and its most extreme form, septic shock. Before arriving at the state of septic shock, the patient has undergone several stages in the continuum of sepsis, starting with a local infection where the pathogen invades the host and releases toxic products, passing the stage where an overwhelming immune response is not only targeted towards the pathogen but also induces morphological damage to cells and tissue leading to organ failure [14]. A faster and more detailed diagnosis could help to save lives in the future. However, currently established microbiological diagnosis involves time-consuming cultivation steps. Thus, a detailed microbiological analysis is only available after 1 day up to several days. Raman spectroscopy is a non-invasive, label-free optical technology that can record in real time the spectroscopic fingerprint of single bacteria, offering a high potential for faster bacterial characterisation directly from patient samples [15–21].

Promising results have been obtained for the diagnosis of UTIs, which account for >150 million infections/year with a spectrum ranging from uncomplicated UTI to life-threatening healthcare-associated sepsis. They are the most frequent infections in women, with >60% of all women having a UTI during their lives. UTIs are also the most common cause of nosocomial infections causing healthcare costs of ca. US$6 billion. The current gold-standard method for pathogen identification from urine culture takes longer than 24 h to give a result [22–24].

To evaluate the potential of Raman spectroscopy for fast and reliable identification of pathogens directly from urine samples, a reference database has been built including the most common causative pathogens, which are *Escherichia coli* (50%), *Klebsiella* spp. (14%), enterococci (10%), staphylococci (6%), *Pseudomonas aeruginosa* (3%) and rarely other bacteria, viruses and fungi. In total, 11 different species have been measured in more than four independent batches per species yielding more than 200 spectra from single cells per species. Fig. 2 displays the averaged Raman spectra per included species. The resulting 2952 spectra were used to train a classification model based on support vector machines. This model was tested with independently measured Raman spectra (in total 514) from the same 11 species yielding a prediction accuracy of 95% [16]. This high accuracy makes the model suitable to be evaluated with real-world patient samples. However, when analysing urine samples from patients, several unknown factors will complicate

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