Mass spectrometric profiling of mucosal metabolome by the DESI-MS of medical swabs – new POC diagnostic approach for infections, dysbiosis and immunological diseases

Nicole Strittmatter, Pamela Pruski, David MacIntyre, Philip Bennett, Trevor Hansel and **Zoltan Takats**

Imperial College London, London, UK, SW7 2AZ

Medical swabs are standard sample collecting devices used in a wide variety of areas of diagnostics ranging from microbiology through early detection of cancer to the non-invasive collection of human cells for genetic testing. These versatile and yet very simple devices are generally sent to the laboratory from the outpatient clinic and processed in a number of potential ways including the smearing of their contents over solid culturing medium for microbiological tests or extracting DNA from the captured cells. Although swabs have been used almost for a century in medicine for sampling (and application or removal), the technique has a number of shortcoming as a means of sample collection, including the poor reproducibility and the loss of spatially resolved information, i.e. if the oral cavity of the patient is sampled using a cotton swab, it is impossible to find out whether the bacteria found on the swab were originated from teeth, gum or a potential ulcer. Likewise, if no pathogenic bacteria were found to grow from the swab, it does not necessarily mean that there were no pathogens at all in the investigated body cavity. Further disadvantage of swab-based diagnostics – which is shared with almost all biofluid-based diagnostic protocols – is the lack of POC capability.

Desorption Electrospray Ionization (DESI) was developed as one of the first ambient mass spectrometry methods more than a decade ago. Although the feasibility of direct skin and direct mucosal sampling was pointed out in the first publications describing the technique, these applications did not gain popularity, mostly due to concerns regarding high voltage of the electrospray probe, use of organic solvents and lack of compatibility with currently used medical diagnostic methods. Present study was aimed at bridging this gap by moving away from the concept of in-vivo sampling and concentrating on the rapid analysis of a standard medical sample, i.e. mucosal swabs.

Methods

XevoG2XS Quadrupole/time-of-flight mass spectrometer (Waters Corp.) equipped with OmniSpray ion source (Prosolia Inc.) was used for the experiments. Standard medical swabs were used either directly or following chemical modification. Chemically modified swabs were prepared by immobilizing 5μm particle size silica based chromatographic packing on the standard swabs using cyanoacrylate adhesive.

Unmodified and modified swabs were used directly for the sampling of pharyngeal, buccal, nasal and urogenital mucosa. Unmodified swabs were directly subjected to DESI analysis, while modified swabs were rinsed in distilled water and dried prior to mass spectrometric analysis. DESI was operated at 5 μL/min flow rate and 4.5 kV high voltage settings. Solvent systems containing water and isopropanol were used throughout the experiments.

Data was acquired as full profiles both in positive and negative ion modes at a nominal resolution of 20,000 FWHM. Spectra were recalibrated and baseline subtracted prior to statistical analysis. Pattern-level identification was performed by creating statistical models using principal component analysis and linear discriminant analysis of data obtained from patients with established clinical diagnosis (training set) and localizing the unknown data points (test set) in the models.

Bacterial marker database was constructed by the automated analysis of bacterial colonies grown on 6 different media using Rapid Evaporative Ionization Mass Spectrometry (REIMS). Dataset was created comprising the 200 clinically most relevant bacterial species, database was validated using 16S rRNA sequencing. Taxonomical markers were obtained by performing ANOVA test followed by Tukey's HSD test on the dataset on different taxonomical levels. Taxon specific markers were found on phylum, class, order, family, genus and species level although not on each level for each species. Markers included such that indicate the presence or absence of a species while others may indicate only presence but not absence. Other markers are of a combinatorial nature.

Results

Mucosal samples were found to show 300-5000 individual spectral features following de-isotoping and removal of adducts. Overall more than 900 peaks were tentatively identified by exact mass, isotope cluster distribution and MS/MS experiments. Identified peaks include

primary metabolites (energy metabolites, amino acids, organic acids, etc.), simple and complex lipids and bacterial secondary metabolites among many others. The primary chemical identification of spectral content already revealed unexpected pieces of information, e.g. urogenital mucosa was found to produce cholesterol sulphate as the most abundant lipid species, while the lipid was not detected on any other mucosal surface in the body.

Chemically modified swabs allowed the clean-up of the sampling devices prior to mass spectrometric analysis. In case of swabs with immobilized octadecyl-silica, aqueous rinsing resulted in 3-16x improvement in the signal to noise ratio of complex lipids, while signals corresponding to more polar constituents (e.g. lactate) completely disappeared from the spectra. Chemical modification/clean-up was found to be useful in case of analysis targeted at well-defined lipid species, e.g. eicosanoid inflammatory mediators.

Detection and identification of infectious agents was performed in case of pharyngeal mucosa. Study group was efficiently separated into pharyngitis and healthy groups using unsupervised statistical analysis. In case of bacterial pharyngitis, Haemophilus, Streptococcus and Staphylococcus were identified successfully and validated by culturing and MALDI analysis of colonies. Streptococcus sp. were also found in case of mostly viral pharyngitis and healthy subjects, however species-level identification clearly separate the pathogenic and commensal Streptococci. Viral infection was efficiently identified and distinctive difference was found between influenza and adenovirus at pattern level, however no species-specific markers were found in case of viruses. Since MS instrument was deployed at the outpatient clinic, the primary aetiology of the infection could be determined in less than 30 s.

Differentiation between healthy and dysbiotic mucosal flora was studied in case of vaginal mucosa, with the long-term intention of diagnosing females with elevated risk for preterm delivery. In case of healthy vaginal microbiome Lactobacillus sp. were found almost exclusively. In case of subjects with documented dysbiosis/history of preterm delivery an overall variety of 18 genuses were identified together with signals associated with fungal pathogens, mostly belonging to Candida. Although it would be speculative to conclude that the DESI-based method solves the problem of stratification regarding risk of preterm delivery, the preliminary results are highly promising.

The technique was also used for the detection of eicosanoid inflammatory mediators in case of allergy patients challenged by different allergens. While originally the allergen was sprayed into the nose of the volunteers, pre-treatment of the swab with the allergen provided similar results. Prostaglandins, especially D2 were successfully detected by DESI from the swabs. Chemically modified swabs were found to give more reproducible results, due to improved overall sensitivity of the method. The kinetics of PG D2 production was also detected by the DESI method, which opens the door to diagnostics and stratification of allergy patients.