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ORIGINAL PAPER

Characterization of surgical aerosols by the compact single-particle mass spectrometer LAMPAS 3

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Abstract A new method is presented using an optical particle counter and the compact mobile laser mass spectrometer LAMPAS 3 for in situ analysis of single particles generated by electrosurgical dissection of biological tissues. The instrumental performance is demonstrated for analysing aerosol particles formed during rapid thermal evaporation of porcine liver and porcine kidney tissues. Particle number concentrations of up to 5,000 particles per cubic centimetre were detected during surgical dissection. Chemical analysis of tissue particles was performed by bipolar time-of-flight mass spectrometry. The application of an online mass spectrometric particle analysis for surgical aerosols is reported here for the first time.

Keywords Mass spectrometry · Single-particle analysis · In situ tissue analysis · Surgical aerosols

Introduction

Mass spectrometric analysis of particles is an important tool for the characterization of ambient aerosols, the determination of particle sources or the assignment of anthropogenic effects on the environment. Besides the field of atmospheric science, aerosol particles influence human health and industrial processes especially through their parameters size, shape, chemical composition and number concentration. Indoor allergic reactions and nosocomial infections of humans on the one hand and faults in nanotechnological processes or in clean room operation on the other hand are examples of the procedural impact of the surrounding particle–gas mixture. Particles are generated in many technical processes, during fossil fuel combustion in industry, motor vehicles, domestic heating and agriculture, influencing outdoor particle concentration and composition. High particle exposures under indoor conditions in private homes, during office work or particle-generating indoor processes are possible. Examples for the latter processes are dust generation in workshops during material treatment or in medicine during surgical interventions. In the case of medical applications, the treatment of biological tissues with surgical lasers or electrosurgical instrumentation produces high numbers of neutral or charged aerosol particles. Since these aerosols contain harmful substances, there have been devices developed for the aspiration of the so-called surgical smoke to improve workplace safety conditions. This aerosol, however, also carries chemical information about the tissues being dissected, which—in principle—can be used for tissue identification, as reported recently. Biological tissue samples were differentiated and identified by the analysis of surgical smoke produced with different surgical dissection methods combined with ion trap and Orbitrap mass analyzers [1–3]. With this method, called REIMS (rapid evaporative ionization mass spectrometry), characteristic phospholipid patterns could be obtained from various biological tissue samples including several types of

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cancerous tissue. In contrast to the present work, no information about the character of the aerosol, such as particle size distribution and chemical particle composition, could be obtained. Further investigations in this field are necessary for a better understanding of the mechanistic aspects and for optimization of the instrumental setup.

For a comprehensive, rapid and reliable analysis of individual aerosol particles, the use of time-of-flight mass analysers with bipolar ion detection are of particular interest because of the large amount of information obtained on a sub-millisecond timescale using these instruments [4, 5]. The online application of such instrumentation enables particle analysis with a minimum loss of analytical information because no sample preparation or storage is required before analysis due to a direct transfer of the particles from ambient atmosphere into the vacuum of the mass spectrometer. These instruments provide the often required high temporal resolution combined with a high level of specificity. These benefits are especially important in quality control, public safety and clinical diagnostics.

The objective of our methodological development was the design of a compact system for in situ particle analysis with improved instrumental parameters compared with our previously reported experimental setup. The present paper describes the features of our small-footprint laser mass spectrometer LAMPAS 3 (laser mass analyzer for particles in the airborne state), and its application during electrosurgical intervention for in situ particle characterization of biological tissues. Besides application in other research fields, such as atmospheric science or homeland security, medical application of the new instrument in surgical operating theatres can provide an advanced description of tissue dissection processes.

Experimental section

Instrumental development

For the expansion of applicational fields of single-particle analysis systems, a reduction of the instrumental size was necessary combined with an improvement of instrumental performance and user friendliness in operation. The general instrumental concept of the new compact laser mass spectrometer LAMPAS 3 is based on that of our earlier LAMPAS 2 systems [6, 7]. A scheme of the instrumental setup and a picture of the final LAMPAS 3 instrument are shown in Fig. 1.

The dimensions of this mobile unit are 152×64×57 cm ($H \times L \times W$) and its weight is about 150 kg. The new system occupies about one third of the volume (~0.5 m^3) of the LAMPAS 2 instrument, and transportation by only one operator is easily possible. All necessary components for a size-resolved determination of particle composition are integrated inside the rack. These components are arranged as compact as possible to reduce the overall size of the instrument. The highly robust instrument is designed for continuous operation under field conditions. An easy-to-use control and security system is integrated to ensure safe operation of lasers, high-voltage supplies and pumping units. Furthermore, a compact electronic and electrical setup was developed for optimal integration into the frame.

Aerosol particles are introduced into the instrument by separating them from ambient air using a differentially pumped inlet system. This system consists of several chambers which are separated by a nozzle, a skimmer and an orifice (Fig. 1). This well-characterized inlet system is also used in the LAMPAS 2 instruments and was therefore chosen for first measurements with the LAMPAS 3 apparatus as described in this paper. As an alternative, an aerodynamic lens system, see, e.g. [8], was constructed and successfully integrated into the existing differentially pumped inlet system. The modular setup of the inlet system allows a quick exchange of inlet units. During measurements, the pressure in the first pumping stage behind the nozzle is about 10 mbar and is maintained by a rotary pump. The second and third stages behind the skimmer and the orifice are pumped by turbomolecular pumps (70 and 250 l/s, respectively). Typical pressures of 10^{-2} and 2×10^{-6} mbar are achieved during normal operation in the second and third pumping stages, respectively. The fore vacuum for both turbomolecular pumps is provided by a diaphragm pump.

Inside the main vacuum chamber of the mass spectrometer (third pumping stage), particles are optically detected by two continuous laser beams (laser wavelength, λ= 532 nm, 75 mW each) with a spacing of 3.3 mm. The particle size is determined through their size-dependent velocity. A new size-optimized optical setup in two levels was built using optical components as described earlier [6]. After particle detection and size determination, an actively triggered UV laser pulse (laser wavelength, λ=337 nm) evaporates and ionizes the detected particle. The focus of the UV laser beam is positioned 1.8 mm downstream from the second detection laser beam. An electronic circuit of the active-trigger system uses the particle velocity to determine the appropriate delay time for firing the UV laser pulse for particle ionization. With this system, improved particle detection efficiency over the complete size range from 200 nm to at least 10 μm is achieved [7, 9, 10].

The focussed UV laser beam (power density, ~5×10^9 W/cm^2) generates positive and negative ions from the detected particle in the centre of the ion source region. This new bipolar two-stage ion source is combined with two

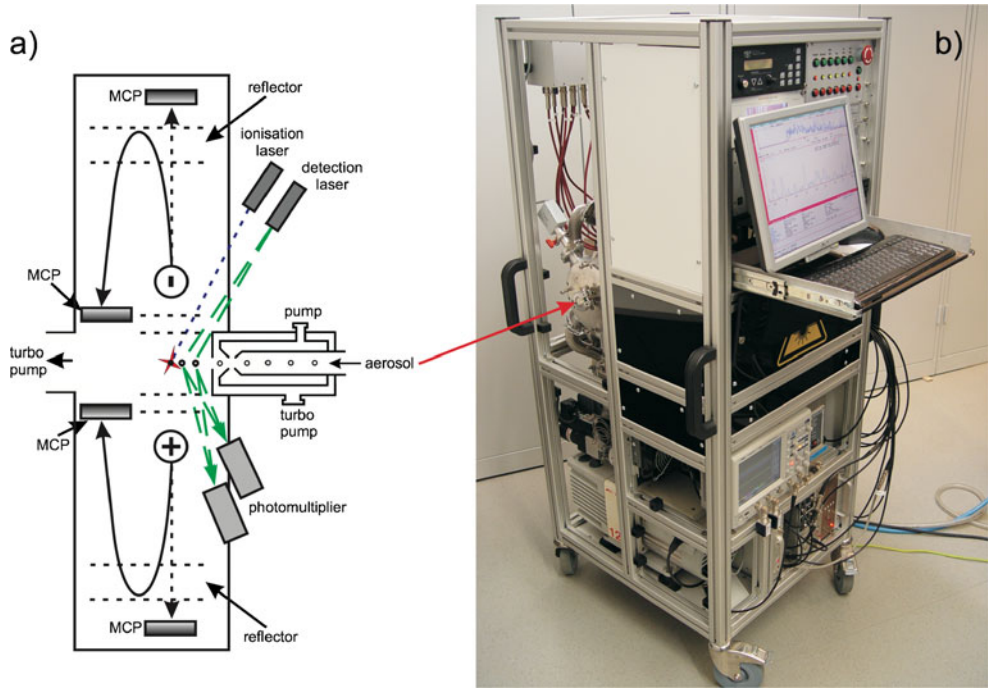

Fig. 1 Compact laser mass spectrometer LAMPAS 3 for the chemical analysis of single aerosol particles: schematic setup (**a**) and picture of the final instrument (**b**)

integrated ion lenses and deflectors for both ion polarities to enhance the number of detected ions.

The new compact vacuum chamber consists of two vertically arranged flight tubes for simultaneous time-of-flight analysis of positive and negative ions [11]. The tubes are prepared for the integration of ion reflectors. Such ion reflectors and an integrated system for delayed ion extraction can optionally be used for an improved determination of ion masses by compensation of the initial ion velocity spread [12]. Using these instrumental options, a significant enhancement of mass accuracy and mass resolving power is possible. These features are important for a reliable and automated assignment of mass peaks in the spectra and for statistical data evaluation.

Ions generated by the UV laser pulse in the centre of the ion source are analysed simultaneously by two independent time-of-flight mass analysers. Flight tube length is 630 mm in reflectron mode and 400 mm in linear mode for both ion polarities. Positive and negative ions are accelerated in a two-stage electrical field in opposite directions by a total accelerating voltage of 5.0 kV for each ion polarity. Ion detection is performed by microchannel plate detectors (MCP) in linear and reflectron modes of operation. Mass resolving power is typically in the range of $M/\Delta M$=400 using the reflectron mode and about 250 in linear mode. An integrated system for data acquisition, data storage and data handling was developed based on a previously described system [13, 14].

Prior to tissue aerosol analysis, the LAMPAS 3 instrument was extensively tested and characterized. For example, a calibration for particle size determination was established and the spectra of single particles of various origins were measured and identified. Examples are included in the Electronic supplementary material (see ESM Figs. S1 to S4).

Experimental configuration

During laser or electrosurgical interventions, large numbers of particles are produced. A laboratory setup was assembled to test the single-particle mass spectrometer LAMPAS 3 for the analysis of tissue-generated particles. Besides the mass spectrometer, the main components of the test setup were a commercially available electrosurgical unit (radioSURG 2200, Meyer-Haake GmbH, Germany) for particle generation from biological tissues and an optical particle counter (OPC type 1.109, Grimm GmbH, Germany) for the determination of particle size distributions. A schematic overview of the setup is shown in Fig. 2.

During experiments, the electrosurgical blade was moved by hand along the tissue surface. Rapid thermal evaporation of the tissue yields gaseous and particulate components. The generated material was aspirated into a home-built housing (size, ~125cm^3) using a Venturi gas jet pump and a 1-m flexible tube (2 mm i.d., polytetrafluoroethylene) [1]. The tube was directly mounted on the hand piece of the cutting electrode. The Venturi housing was connected to the optical particle counter and the LAMPAS 3 instrument employing flexible tubes (TYGON®-R3603, 8 mm i.d.) with lengths of 50 and 200 cm, respectively. Both tubes were placed perpendicularly to the outlet of the Venturi pump. For safety reasons, an appropriate venting system was used to prevent inhalation of the aerosol.

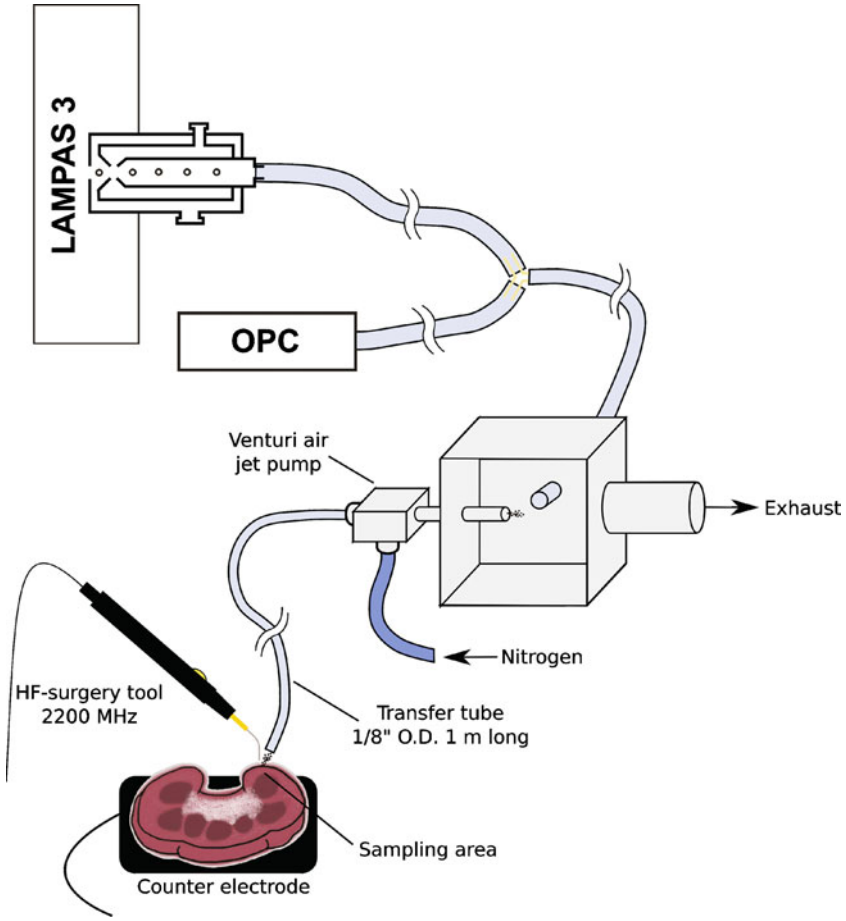

Fig. 2 Experimental setup for the analysis of tissue particles generated by an electroscalpel. The produced particles are transferred to an optical particle counter (*OPC*) for the determination of the particle size distribution and to the inlet of the single-particle mass spectrometer LAMPAS 3 for chemical particle analysis using a Venturi air jet pump

Typically, a nitrogen flow of 20 L/min was used for the operation of the Venturi pump. The electrosurgical unit was used in monopolar cutting mode with a power output of 60 W maximum.

The optical particle counter was used to determine the particle number concentration in 31 size channels in a range between 250 nm and 32 μm. During all measurements, a storage interval of 6 s was chosen.

Several experiments were performed to characterize particle generation from the tissues and to optimize particle detection and analysis with the LAMPAS 3 mass spectrometer. Both mass analysers for positive and negative ions were operated in linear mode. Porcine liver and kidney were used as test samples. Tissues were dissected in 20 periods with a total analysis time of 87 min and a typical cutting duration of about 5 min. After a first test of the system, three main episodes were completed to acquire data during cutting of porcine liver (two episodes) and porcine kidney. Alternatively to the Venturi pump-based setup, the generated aerosol was also directly transferred to the OPC and LAMPAS 3 to investigate the influence of the Venturi air jet pump. This procedure resulted in the immediate clogging of the inlet of the mass spectrometer and an overloading of the OPC.

Results and discussion

During cutting of the biological tissues by the electrosurgical unit, the particle mass spectrometer LAMPAS 3 and the OPC were operated in parallel to register mass spectra from single particles and to measure size-resolved particle number concentrations. An example of particle number concentrations for selected particle size ranges during an experimental period of about 5 min is shown in Fig. 3.

The diagram shows that whilst the particle production fluctuates significantly during 5 min of electrosurgical tissue evaporation, the particle size distribution is largely independent from the actual particle number. Only the onset and offset sections of the curves show significant differences due to the different velocities of particle size fractions in the instrument. The fluctuation of particle number values was associated with the inhomogeneous nature of tissue and variation of the dissection depth.

The size-resolved number concentration averaged for the three main episodes is displayed in Fig. 4. The figure clearly illustrates that the particle size distribution is also widely independent of the biological origin of the tissue and certain experimental conditions. The two episodes, called “kidney” and “liver 3”, were performed using a

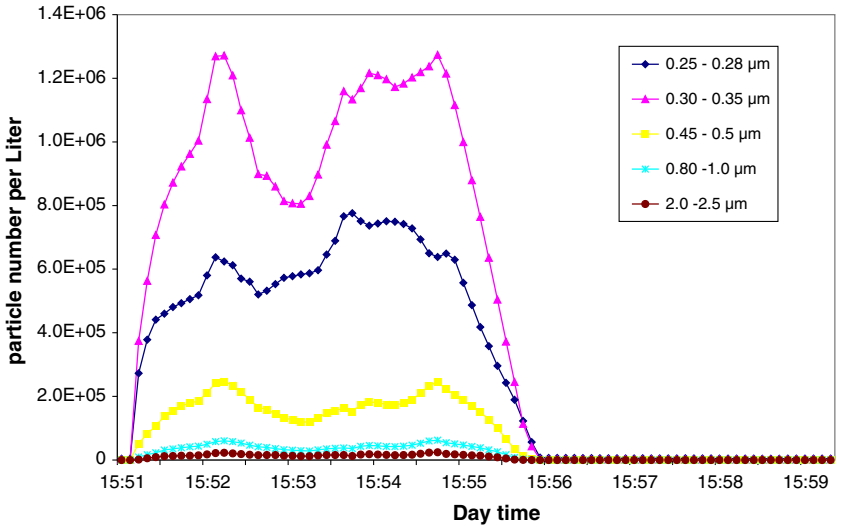

Fig. 3 Size-resolved particle number concentrations determined by OPC during the electrosurgical dissection of porcine liver tissue

Venturi pump for aerosol transfer. Nevertheless, particle size distributions were very similar for the three episodes and only slight deviations were observed. This indicates that particle production with respect to size is determined by the evaporation process and that neither the biological nature of the tissue nor the means of aerosol transport has a significant influence on this process. The similarity of curves extends to the formation of local maxima at sizes of 300 and 600 nm. The reason for this observation has to be investigated in further experiments.

More than 1,200 single-particle spectra were registered during the three experiments. The numbers of single-particle mass spectra detected in size bins of 100 nm are shown in Fig. 5. For all periods, the highest number of spectra was detected in the size range of 400 nm; a slightly increased number of spectra at 600 nm was observed. This is in good agreement with the relative number concentrations determined with the OPC in these two sizes. During the experiment "liver 2", a higher number of spectra was recorded for particle sizes above 1.2 µm in diameter in comparison with the two other main periods. During the "liver 2" period, the Venturi pump was not used and particles of all sizes were directly transferred to the inlet of the LAMPAS 3 system. Using the Venturi pump, larger particles with higher inertia were transferred to the LAMPAS 3 instrument with a reduced efficiency due to a preferred flow direction perpendicular to the inlet tube inside the Venturi housing (see Fig. 2). The reduced number of spectra in the size range below 400 nm in diameter was associated with the lower transmission efficiency of smaller particles in the inlet system [15]. Using an aerodynamic lens optimized for this size range could enhance the detection efficiency of these particles.

Basically, two distinctively different main types of pairs (positive–negative) of mass spectra were detected (shown in Figs. 6 and 7), corresponding most likely to two

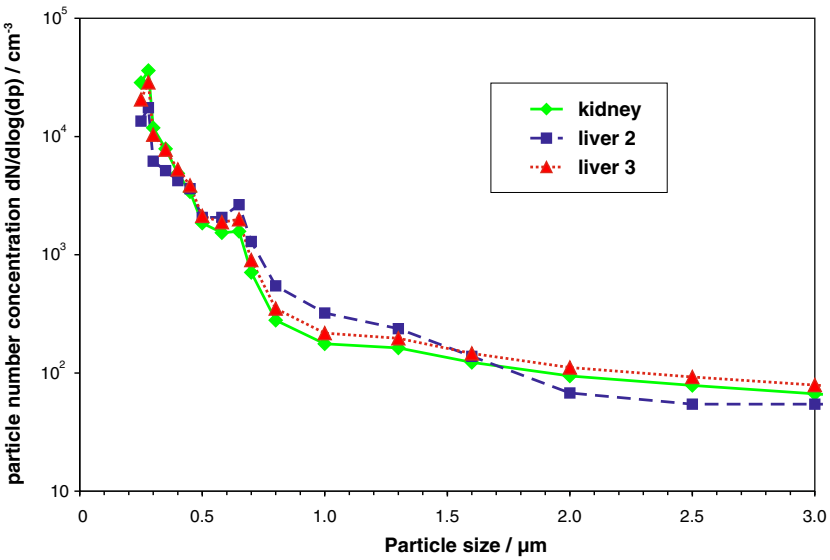

Fig. 4 Size-resolved particle number concentration averaged over all measuring times of three cutting episodes

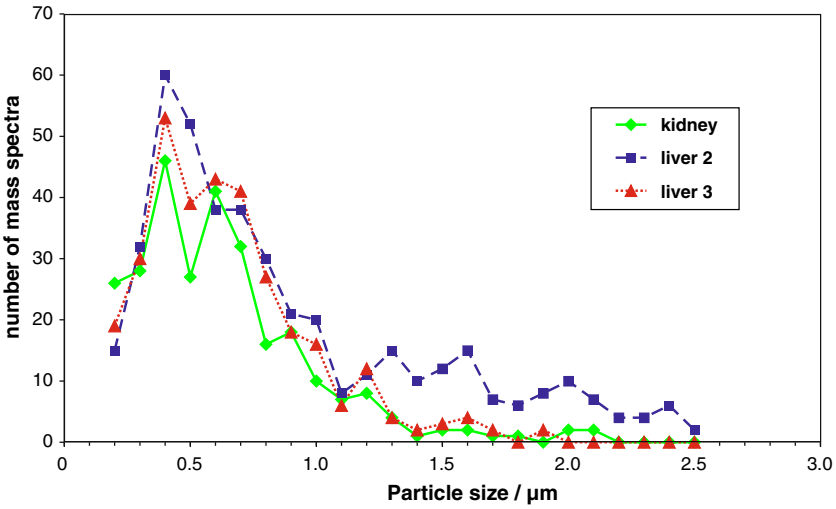

Fig. 5 Size-resolved number of single particle mass spectra detected during the main cutting periods for porcine liver and kidney

considerably different types of particles. The spectra shown in Fig. 6 feature predominantly carbon cluster ions (C_n^-/C_n^+, n=2–19, m/z=24, 36, 48…) in the lower mass range, as is expected based on the high carbon content of biological tissue. The negative ion spectrum additionally contains anion signals of $CN^-/C_2H_2^-$ (m/z=26) and nitrate ions (NO_2^-, m/z=46; NO_3^-, m/z=62). Further characteristic signals in the positive ion spectrum correspond to sodium (Na^+, m/z=23), potassium (K^+, m/z=39) and ammonium (NH_4^+) ions.

In the higher mass range, intensive signals were detected, especially in the negative ion mode. These species were associated with the thermal degradation products of carbohydrates, lipids, amino acids and proteins. The observation of carbon cluster ions in the lower mass range is probably the result of the laser ablation process due to the

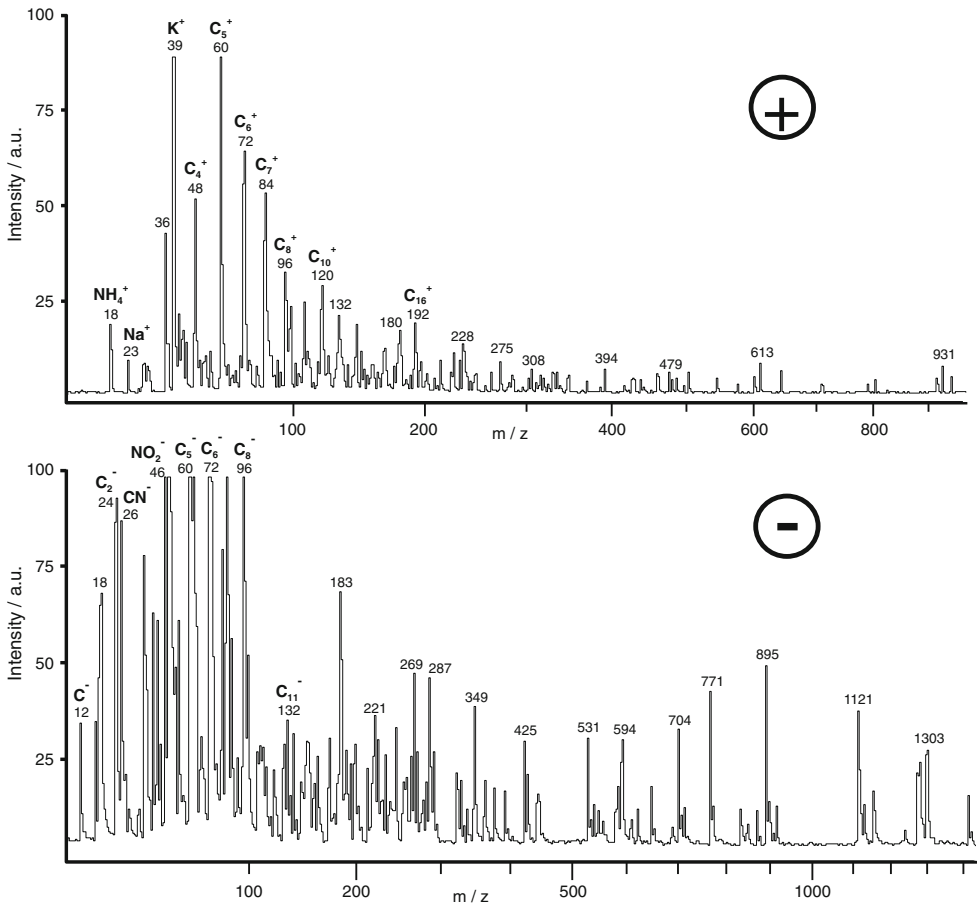

Fig. 6 Positive ion and negative ion mass spectrum of a single particle generated by electrosurgical dissection of porcine liver tissue (aerodynamic particle size, ~0.9 µm)

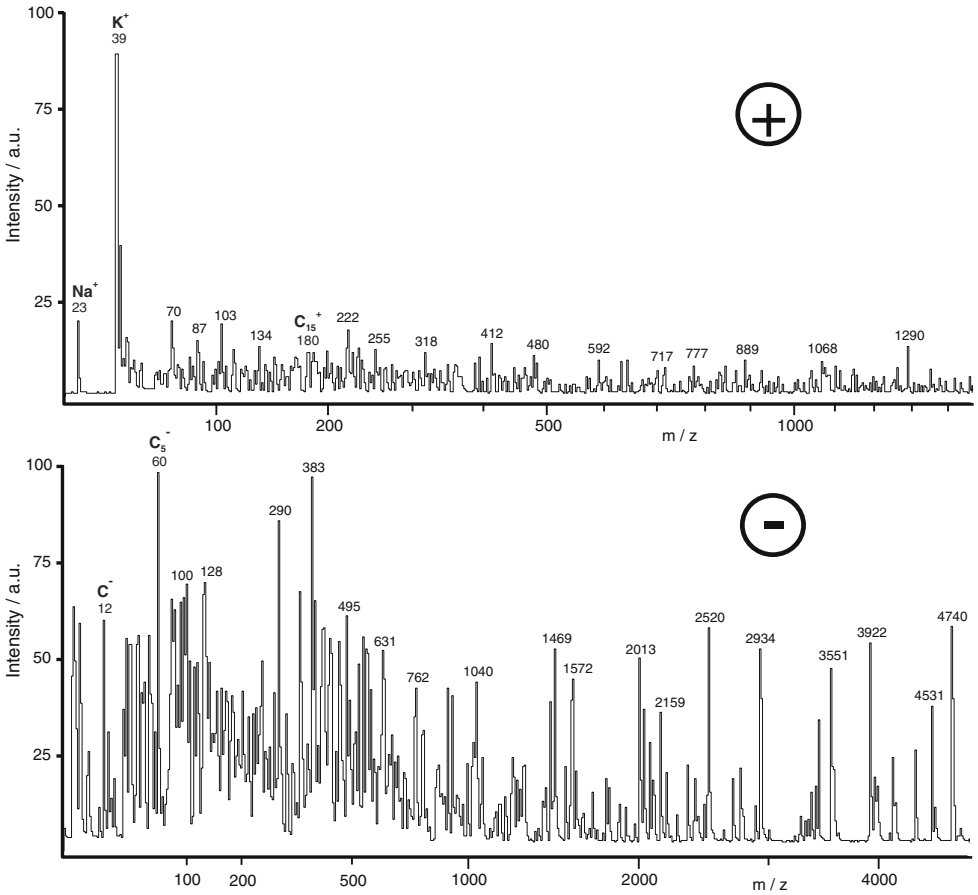

Fig. 7 Positive ion and negative ion mass spectrum of a single particle generated by electrosurgical dissection of porcine liver tissue (aerodynamic particle size, ~0.3 μm)

high power density of about 10^9 W/cm^2 in the UV laser focus.

In comparison to Fig. 6, the spectra in Fig. 7 look considerably different. The carbon cluster ions are almost absent; however, ion signals can be observed up to m/z= 4,000 and above in the negative ion spectrum. The positive ion spectrum of Fig. 7 also shows a dominant potassium ion signal.

The electrosurgical tissue evaporation comprises the Joule heating of tissues by electric current, followed by partial denaturation of the macromolecular components and boiling of the water content. The process may also involve the carbonization and effective burning of tissue material, especially at high dissection power settings. Since both boiling of aqueous tissue material and carbonization/ burning of dried residues yield large amounts of aerosol, one can expect at least two types of particles. Whilst boiling produces aqueous droplets containing dissolved organic and inorganic species, carbonization/burning results in the formation of carbonaceous solid particles with large amounts of adsorbed species on the surface. Although there is no direct evidence for this assumption, we have associated spectra, as shown in Fig. 7, with droplet-type aerosols and spectra, as shown in Fig. 6, with carbonaceous particles. This assumption explains the presence (or absence) of carbon clusters and also the signals corresponding to macromolecular species.

The spectra of both types as shown in Figs. 6 and 7 were recorded very often during the experiments, but no unambiguous spectra patterns or significant marker ions for a certain type of tissue were found in these first studies. The observation of ions in the mass range above m/z=500 is unusual in the online mass spectrometry of inorganic particles because of the high laser power densities. In specific online applications of single-particle mass spectrometry for the identification of bioaerosols such as bacteria, spores and viruses, the mass range for the analysed components could be extended up to m/z=20,000 [16–19]. Further investigations and optimization of the reported method for particle analysis of biological tissues are planned for an improved characterization of electrosurgical processes and particle and ion formation mechanisms, e.g. by reducing the used laser irradiance.

Conclusion

The presented results demonstrate the ability of online single-particle mass spectrometry for the fast analysis of biological tissues. The application of electrosurgical equip-

ment on tissues generates a large number of particles in the size range above 250 nm, accessible for such instruments. The compact laser mass spectrometer LAMPAS 3 was successfully applied to tissue particle analysis and can be operated under operating room conditions. Ions could be detected in a broad mass range, reflecting the great variety of bioorganic compounds present in biological tissues. The results are promising for future experiments to use chemical information of particles generated during tissue dissection to better understand the electrosurgical process.

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