Mass Spectrometry

DOI: 10.1002/anie.200902546

In Vivo, In Situ Tissue Analysis Using Rapid Evaporative Ionization Mass Spectrometry**

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The analysis of intact biological tissues by mass spectrometry (MS) has been pursued for more than three decades. However, mass spectrometric methods have always put strong constraints on the geometry and the preparation of these samples. Even with the recent advent of ambient ionization methods, not all of these restrictions have been lifted.[1–5]

MS analysis of biomolecules in tissue has traditionally been achieved by desorption ionization methods including secondary ion mass spectrometry (SIMS),[6–11] matrix-assisted laser desorption (MALDI),[12–17,19,20] and desorption electrospray ionization (DESI)[4,5,18] methods.

While desorption ionization methods are not appropriate for the analysis of vital (living) tissues, rapid thermal evaporation has the potential to establish the in situ, in vivo ionization of tissue constituents. The possible formation of organic ions from condensed-phase samples in a purely thermal process was initially proposed by Holland et al.,[21] and it was successfully demonstrated later.[22–24] The rationale of rapid heating was to achieve molecular evaporation rates comparable to the rate of decomposition, which results in the formation of a considerable quantity of gaseous molecules or molecular ions.

The quest for efficient thermal evaporation methods has led to the development of various thermally assisted ionization methods, including thermospray ionization.[25] Since collisional cooling of nascent ions at higher pressure is more effective, thermal evaporation at atmospheric pressure is expected to suppress thermal decomposition. Atmospheric pressure thermal desorption ionization was demonstrated recently by the desorption of organic cations with minimal thermal degradation.[26,27]

The present study is based on the discovery that rapid thermal evaporation of biological tissues yields gaseous molecular ions of the major tissue components, for example, phospholipids. As thermal evaporation of tissues is widely used in surgery (i.e., electrosurgery and laser surgery), it was sensible to use dedicated surgical instruments for the experiments. Combination of surgical and MS techniques also offers a possibility for in situ chemical analysis of tissue during surgery. Since the key feature of the technique is the fast evaporation of a sample, it was termed "Rapid Evaporative Ionization Mass Spectrometry" (REIMS). The tentative mechanism of ion formation is described in the Supporting Information.

Electrosurgical dissection is based on the Joule heating and evaporation of tissues by an electric current. The presence of ionized water molecules during electrosurgical dissection raises the possibility of an alternative ionization mechanism involving neutral desorption and chemical ionization in the gas phase. For more details, see the Supporting Information. An electrosurgical electrode was used as an ion source coupled to a distant mass spectrometer employing a Venturi gas jet pump and 1–2 m long polytetrafluoroethylene (PTFE) tubing (Figure 1).[28]

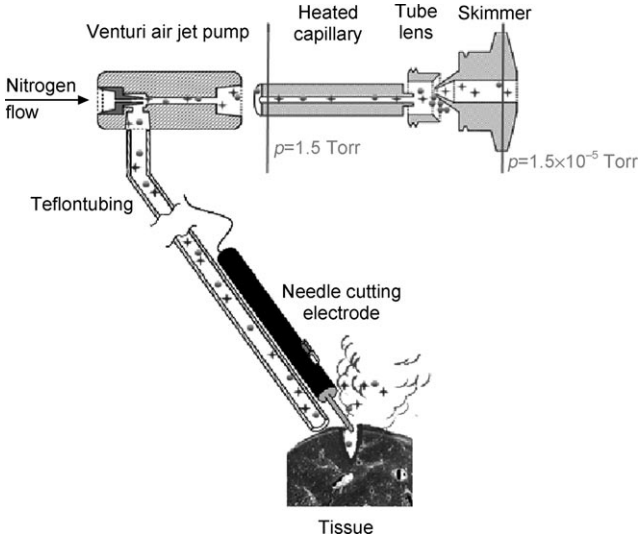

Figure 1. Experimental setup for REIMS tissue analysis. Tissue is evaporated along the contact surface of the surgical electrode. The ions are transferred to the mass spectrometer using a Venturi pump. Positive and negative ions are produced equally; ions are separated by polarity in the tube-lens/skimmer region. Dots represent neutral particles, and stars represent charged particles.

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[**] Supported by the Hungarian National Office for Research and Technology (Nanodrug Grant) and the European Research Council (ERC-STG 210356).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200902546.

REIMS analysis of vital porcine liver yields mass spectra featuring ions mainly in the m/z 600 to 1000 mass range in both polarities (Figure 2). The signals were identified by accurate mass measurements and MS/MS experiments as

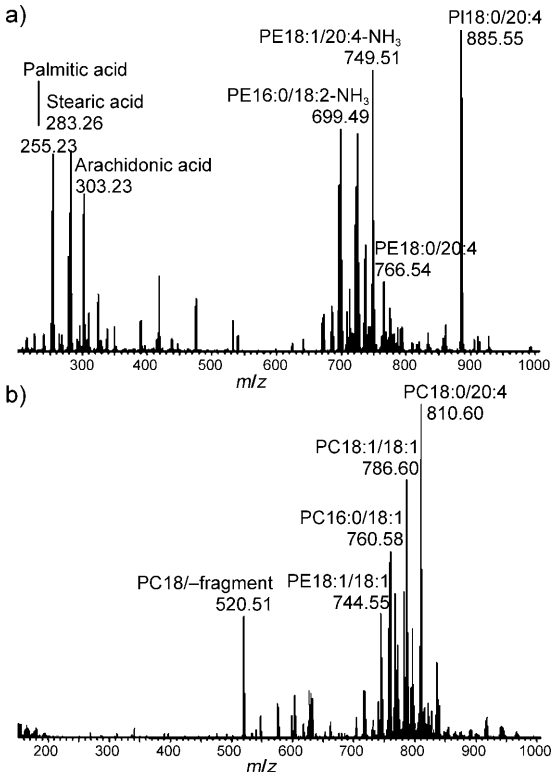

Figure 2. REIMS spectra of vital porcine hepar tissue in a) negative-ion and b) positive-ion mode. Signals were assigned by exact mass analysis. See Table 1

glycerophospholipids or their thermal degradation products. The fact that phospholipid ions dominate the spectra was tentatively associated with their high abundance in the tissues, their ionic character under physiological conditions, and their low desolvation (dehydration) enthalpy (see the Supporting Information). The identified lipids are listed in Table 1.

Different tissues were found to yield characteristically different spectra. Hence, a tissue identification system was developed using a spectral library and principal-component analysis (PCA). An alternative tissue identification algorithm was also developed (for details, see the Supporting Information). The data points obtained by cutting through a porcine kidney were analyzed by PCA and were plotted as a function of the first two PCA parameters. Spectra from the cortex, medulla, and pelvis were clearly separated (Figure 3). Since the timescale of REIMS analysis of the tissues is in the range of 0.1–0.3 s and data analysis takes 0.1–0.15 s, the system provides virtually real-time information on the nature of the tissue being dissected.

The significance of in situ, real-time tissue identification capability lies in its potential use in cancer surgery. Cancer cells possess different phospholipid compositions than normal cells.[29–31] In situ identification of malignant tumors by

Table 1: Identified lipids and degradation products in the negative and positive ion mode REIMS spectra of vital porcine liver tissue. (For MS data used for identification, see the Supporting Information; number of C atoms:degree of unsaturation.)

Compound	Fatty acids
Positive ion mode	
phosphatidyl cholines (PC)	16:0,16:1,18:1, 18:2, 20:4,20:2, 20:3 22:6
phosphatidyl ethanolamines (PE)	14:0, 16:0, 16:1, 18:1, 18:2, 20:4, 20:2, 20:3 22:6
sphingomyelins (SM)	18:0, 18:1, 20:4, 22:6
triglycerides (NH_4^+ adducts)	16:0, 16:1, 18:1, 18:2, 20:4, 20:2, 20:3 22:6
Negative ion mode	
fatty acids	2:0, 3:0, 4:0, 8:0, 8:1, 10:0, 10:1, 12:0, 12:1, 14:0, 14:1, 16:0, 16:1, 18:0, 18:1, 18:2, 20:2, 20:3, 20:4, 22:6
phosphatidyl ethanolamines (PE)	14:0, 16:0, 16:1, 18:1, 18:2, 20:4, 20:2, 20:3 22:6
phosphatidyl ethanolamines -NH_3 (PE-NH_3)	identical to PE
phosphatidyl serines	16:0, 18:1, 18:0
phosphatidyl inositols (PI)	16:0, 18:0, 20:4
sulfatides	16:0, 18:1, 20:4
plasmalogens	16:0, 18:1
phosphatidic acids	16:0, 18:1, 18:0

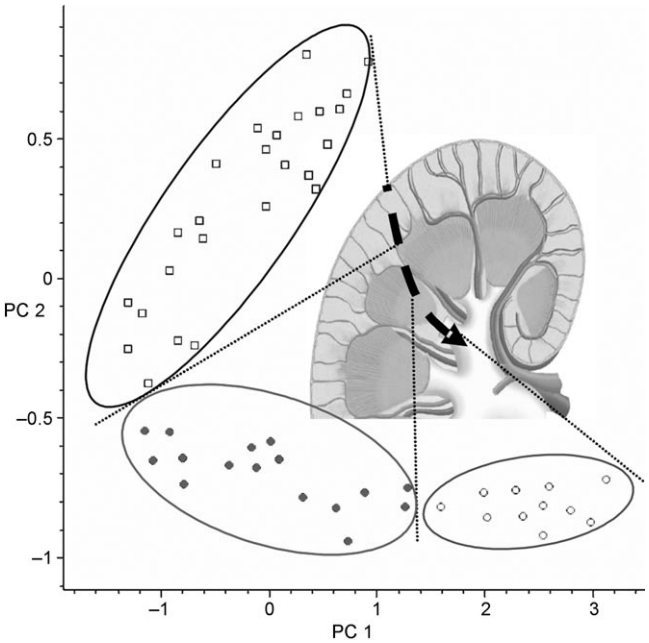

Figure 3. Two-dimensional principal-component analysis plot of porcine renal REIMS spectra. The arrow indicates the direction of transection; spectra were recorded continuously. □: cortex, ●: medulla, ○: pelvis.

REIMS was tested on a canine melanoma model. The REIMS spectra of the melanoma and the infiltrated lymph node are quite similar, while both are considerably different from the healthy epithelium (Supporting Information, Figure S4). REIMS analysis reveals not only the presence of

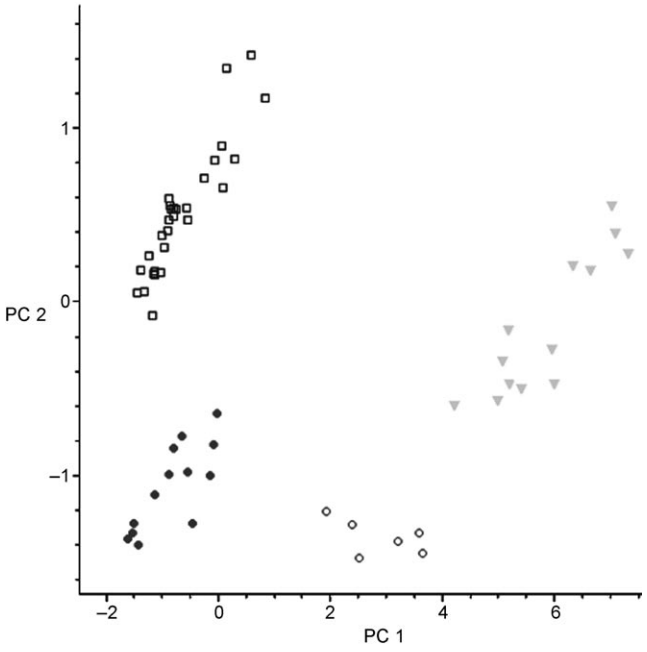

Figure 4. Two-dimensional principal-component analysis plot of REIMS spectra from healthy and cancerous breast tissue. □: in situ low grade carcinoma, ●: mast cell tumor (grade II–III), ○: necrotic mast cell tumor tissue, ▼: healthy tissue.

malignant tumor tissue but also provides information on the grade and the possible necrosis of tumors (Figure 4).

The presented results serve as a basis for future development of surgical methods guided by mass spectrometry. REIMS allows rapid analysis of vital and processed tissues and real-time identification of tissue features during surgery. Application of the method for the localization of malignant tissue (including metastases) during tumor resection was successfully demonstrated. Since all in vivo experiments were performed under conditions required in human surgical facilities using approved instruments (with the exception of distant MS), the methods can directly be utilized in operating theaters.

Experimental Section

A commercially available electrosurgical unit (ICC 300, Erbe Elektromedizin GmbH) was used for ionization and tissue dissection in cutting mode 4. The maximum cutting power was set to 80 W and the electrosurgical handpiece was equipped with a vent line (Erbe). The smoke vent line was connected to a VAC 100 Venturi pump (Veriflo, Parker Instruments) using $^1/_8''$ OD 2 mm ID PTFE tubing. The Venturi pump was driven by 20 L min^{-1} air flow. The pump exhaust was directed towards the atmospheric inlet of the mass spectrometers. High-resolution mass spectrometry was performed using a Thermo LTQ Orbitrap Discovery instrument, and a Thermo LCQ Deca XP instrument was used for in vivo experiments.

Received: May 13, 2009
Revised: August 8, 2009
Published online: September 10, 2009

Keywords: biomarkers · cancer diagnostics · mass spectrometry · phospholipids · tissue analysis

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