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Direct identification of proteins from T47D cells and murine brain tissue by matrix-assisted laser desorption/ionization post-source decay/collision-induced dissociation

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The purpose of this study is to determine the feasibility of the direct matrix-assisted laser desorption/ionization (MALDI) identification of proteins in fixed T47D breast cancer cells and murine brain tissues. The ability to identify proteins from cells and tissue may lead to biomarkers that effectively predict the onset of defined disease states, and their dynamic behavior could be an important hint for drug target discoveries. Direct tissue application of trypsin allows protein identification in cells and tissues, while maintaining spatial integrity and intracellular organization. Using a chemical printer, matrix was co-registered on trypsinized human T47D breast cancer cells and cryo-preserved sections of murine brain tissue, followed by MALDI post-source decay (PSD) or MALDI collision-induced dissociation (CID), respectively. Mass-to-charge (*m/z*) data from the cells and brain tissues were processed using Mascot© software interrogation of the National Center for Biotechnology Information (NCBI) database. Histone H2B was identified from cultured T47D human breast cancer cells. Tubulin β2 was identified from mouse brain cortex following an induced stroke. These results suggest that MALDI PSD/CID, combined with bioinformatics, can be used for the direct identification of proteins from cells and tissues. Refinements in preparation techniques may improve this approach to provide a tool for quantitative proteomics and clinical analysis. Copyright © 2007 John Wiley & Sons, Ltd.

The purpose of this study was to determine the feasibility of direct cellular matrix-assisted laser desorption/ionization (MALDI) identification of proteins in fixed cells and tissues. The MALDI mass spectrometry (MS) modes used in this study were post-source decay (PSD)[9,10,16,17] and collision- induced dissociation (CID).[18] The direct identification of biomarkers in tissue and body fluids would allow early diagnosis, monitoring of disease progression, and stratification of patients into subgroups that respond better to different types of treatment. However, cellular identification is necessary to determine the origin of these proteins, because non-specific protein changes can be observed in body fluids as a non-specific reaction to ischemia, inflammation, or neoplasm. Since 20–25% of all intracellular and extracellular domain membrane-bound proteins are secreted into body fluids and the general circulation, tissue identification of proteins may lead to the identification of the same or similar proteins in other body fluids such as serum, urine, saliva, and stool.[1] The specific localization of these proteins within the cytoplasm, nucleus or intracellular organelles may elucidate metabolic pathways and identify specific drug targets.[2] There have been numerous descriptions of direct protein identification from lysed cells.[3–10] Direct tissue identification of proteins using non-fixed, lysed or desiccated tissues has been reported in other studies.[11–15] In all these studies, various forms of cellular disruption including cell lysis, desiccation or autolysis resulted from the different preparation techniques. Thus, intracellular anatomic relationships were disrupted and specific loci of protein origin were lost. In this study, cellular integrity was maintained by the specific techniques of cryoprotection and instant cellular and tissue fixation.

This preliminary report describes a new method of direct protein identification from cells and tissue in T47D breast cancer cells and stroke brain tissue from a murine stroke model. The advantages of the method are the relative ease and speed of

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tissue preparation for protein analysis. This should encourage early migration of direct tissue MALDI from the laboratory to clinical practice where it may become a basis for identification of protein molecular biomarkers of clinical disease.

EXPERIMENTAL

All studies were performed using protocols approved by the New York University Institutional Review Board (IRB) and Institutional Animal Care and Use Committee (IACUC). Calibrants and matrix chemicals were purchased from Sigma (St. Louis, MO, USA), Invitrogen (Carlsbad, CA, USA), Sakura Finetek (Torrance, CA, USA) and Scott Specialty Gases (Plumsteadville, PA, USA), and were used without further purification.

Instrumentation

T47D breast cancer cells were analyzed using an Axima-CFR^{TM}*plus* MALDI-TOF mass spectrometer (Kratos/Shimadzu Biotech, Manchester, UK) in reflectron mode MALDI MS and MALDI PSD (mass range *m/z* 10–30 000). Ischemic murine brain tissue was analyzed in linear mode MALDI MS, with an Axima-CFR+^{TM} (mass range *m/z* 10–200 000), and in reflectron mode MALDI MS and MALDI CID, with an Axima-QIT^{TM} MALDI TOF/TOF mass spectrometer (mass range *m/z* 10–5000) (Kratos/Shimadzu Biotech). Prior to the analysis of peptides in reflectron mode by MALDI MS and MALDI PSD, the Axima-CFR^{TM}*plus* was externally calibrated using a four-point calibration with bradykinin fragment 1–7 ($[M+H]^+_{mono} = 757.39$), angiotensin II ($[M+H]^+_{mono} = 1046.54$), synthetic peptide fragment ion $P_{14}R$ ($[M+H]^+_{mono} = 1533.85$), and ACTH fragment 18–39 ($[M+H]^+_{mono} = 2467.19$). Prior to the analysis of peptides in reflectron mode by MALDI MS and MALDI CID, the Axima-QIT^{TM} was externally calibrated with the synthetic peptide fragment ion $P_{14}R$ ($[M+H]^+_{mono} = 1533.85$). Prior to the analysis of intact proteins in linear mode, the Axima-CFR^{TM}*plus* was externally calibrated using a three-point calibration with cytochrome C ($[M+H]^+_{average} = 12361.9$), aldolase ($[M+H]^+_{average} = 39212.28$), and serum albumin ($[M+H]^+_{average} = 67430.09$). The Axima-CFR^{TM}*plus* calibrated mass accuracy for PSD was ±0.5 *m/z* unit. The Axima-QIT^{TM} calibrated mass accuracy for MS/MS was 50 ppm. All linear and reflectron mode spectra were acquired with a 337-nm N_2 laser. The laser beam spot size of both the Axima-QIT^{TM} and Axima-CFR^{TM}*plus* could be focused from 50 to 150 μM.

Breast cancer cell preparation

Human breast cancer cells (T47D) were grown to 70% confluence in Dulbecco's modified Eagle's medium (Invitrogen), containing 10% fetal bovine serum (FBS) (Invitrogen) in six Petri dishes, with approximately 10^6 cells per dish. Cell counts were determined with a hemocytometer. The cells appeared microscopically intact after washing with phosphate-buffered saline (PBS). The media were aspirated and the cells washed with PBS. The cells from each of the Petri dishes were scraped into five Eppendorf tubes. The cells were treated according to five different protocols: (1) 0.5% polyvinylpyrrolidone (PVP 360) in 60% methanol, washed with 60% methanol to remove the PVP 360; (2) 0.5% PVP 360 in 95% ethanol; (3) 0.5% polyethylene glycol sorbitan monolaurate (Tween 20) in 60% methanol; (4) Tween 20 in 95% ethanol; (5) distilled water on ice for 5 min which caused cell lysis. The cells appeared microscopically intact after treatment with protocols 1–4. All studies were repeated three or more times.

Breast cancer cell MALDI analysis pre-trypsin treatment

A 1.5 μL aliquot of 60% methanol fixed cell samples (approximately 10^5 cells) from each of the five protocols was spotted onto each of 12 wells (N = 12) on a cooled conductive metal plate, and allowed to air-dry at room temperature. The matrix, α-cyano-4-hydroxycinnamic acid (CHCA), 10 mg/mL in 50:50 acetonitrile (ACN)/0.1% trifluoroacetic acid (TFA) in water, 40 000 pL (total/well with multiple printings), was robotically printed over the cells with a chemical printer (ChIP 1000, Shimadzu Corp., Kyoto, Japan) and allowed to crystallize at room temperature. The computer-controlled robotic chemical printer can deliver precise pL volumes at predetermined loci. The raster configuration can be predefined for co-registration of solutions, trypsin or matrix in this study, and also transferred to the networked mass spectrometers. All printed microdots were co-registered by the printer, and the printing raster transferred to the networked mass spectrometer. This ensured that the MALDI laser raster was aimed precisely at the same loci where matrix had been applied, and thus facilitated automated data acquisition. MALDI MS analysis was performed in reflectron mode (accelerating voltage 20 kV, reflectron voltage 25 kV). Each spectrum represents the cumulative average of 1000 profiles from each well.

Breast cancer cell MALDI analysis post-trypsin treatment

Porcine trypsin (Promega, Madison, WI, USA), 0.2 mM in 25 mM ammonium bicarbonate buffer (pH 8–8.5), was added to each of the five samples. The five closed Eppendorf tubes were placed in a 95% humidified incubator at 37°C for 90 min. A 1.5 μL aliquot of 60% methanol fixed trypsinized cells (approximately 10^5 cells) from each of the five samples was spotted onto each of 12 wells (N = 12) on a cooled conductive metal plate and allowed to air-dry at room temperature. The matrix, CHCA, 10 mg/mL in 50:50 ACN/0.1% TFA in water, 40 000 pL (total/well with multiple printings), was robotically printed over the trypsinized cells in each well with a ChIP 1000 chemical printer and allowed to crystallize at room temperature. The printer co-registered all printed microdots, and transferred the printing raster to the networked mass spectrometer as was done with the non-trypsinized samples. Analysis was performed in reflectron mode, and MALDI MS and MALDI PSD spectra were obtained (accelerating voltage 20 kV, reflectron voltage 25 kV). Each spectrum represents the cumulative average of 1000 profiles from each well.

Stroke brain tissue analysis – stroke brain preparation

Six adult Swiss murine brains were studied 24 h after induction of ischemic strokes with a non-invasive photo-

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chemical murine stroke model using Rose Bengal, a fluorescein dye, and visible light.[19,20] A photochemical reaction between the circulating Rose Bengal and the light produces singlet oxygen that begins a chemical cascade resulting in a focal stroke. After Rose Bengal had been injected into the peritoneum, an infarct was induced in the right parietal cortex by a light applied via a cold fiber optic light pipe held against the scalp overlying the right parietal cortex. The left parietal cortex received no light exposure, and was the control hemisphere.

After 24 h, the mice were anesthetized with Isoflurane 1% (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoroethane), a halogenated volatile anesthetic which induces and maintains general anesthesia by depression of the central nervous system and resultant loss of consciousness (Isoflurane, Scott Specialty Gases), and infused with 5% sucrose for cryoprotection by percutaneous transthoracic left ventricular injection.[21] Under deep terminal anesthesia, the brain was removed rapidly, and immersed in solidifying isopentane cooled by liquid nitrogen. The frozen brain was affixed to the cryomicrotome chuck with a few drops of ice-water (specimen temperature −15°C, microtome blade temperature −35°C).

Three consecutive, 12 μm brain tissue cryosections were obtained from each of the six animals with stroke brains (N = 6). The first section was applied to a conductive metal plate for pre-tryptic digestion MALDI MS. The second section, essentially a mirror section of the first section, was applied to a conductive metal plate for post-tryptic digestion MALDI MS. The third section from each animal was applied to a cooled standard glass slide for histological study. Immediately following application of a section to a cooled conductive metal plate, it was rapidly immersed in 60% methanol for fixation. Likewise, immediately following application of a section to a glass slide, it was rapidly immersed in 60% methanol for fixation.

Stroke brain tissue MALDI analysis pre-trypsin treatment

The laser power was tuned to maximize signal intensity for both linear and reflectron mode. Laser power and flux, and accelerating and pulse extraction voltages were held constant across all the samples during data acquisition; with data uniformity assured by employing automatic acquisition. The matrix used for linear mode MALDI MS protein spectra identification, CHHA (10 mg/mL in 50:50 ACN/0.1% TFA in water: 40 000 pL), was applied with the same chemical printer to each of five equidistant loci across the left non-stroke hemisphere, and to six equidistant loci across the right stroke hemisphere. The print spot size was consistently less than 150 μm for matrix (microscopically verified). MALDI MS in linear mode, (accelerating voltage 20 kV) was used to analyze five sampling points in the normal hemisphere, and six points in the right stroke hemisphere, including the unaffected cingulate gyrus. Each spectrum represents the cumulative average of 400 profiles from each brain locus.

Stroke brain tissue MALDI analysis post-trypsin treatment

Porcine trypsin was printed on these similar loci on the second, mirror, brain section, from all six stroke brains. Tryptic digestion was continued in a 95% humidified incubator for 90 min at 37°C, and the sections air-dried at room temperature. Matrix, 2,5-dihydroxybenzoic acid (DHB), 10 mg/mL in 50:50 ACN/0.1% TFA in water, was printed on the same trypsin loci and allowed to crystallize at room temperature. DHB was chosen for use in the MALDI CID application because there was less fragmentation of the precursor ion in MS mode (manufacturer's recommendation). The combined trypsin/matrix spots did not exceed 200 μm in diameter (microscopically verified). MALDI MS and MALDI CID spectra in reflectron mode (accelerating voltage 50 V, reflectron voltage 2 kV) were obtained from these similar loci. Each spectrum represents the cumulative average of 400 profiles from each brain locus.

The T47D breast cancer cell and murine stroke brain tissue protocols are summarized in Table 1.

Data processing

Individual peptide product ion *m/z* values obtained from the MALDI MS, MALDI PSD and MALDI CID spectra were used in the Mascot© software program (public server) to interrogate the National Center for Biotechnology Information (NCBI) database. Protein identifications with probability scores and corresponding *p*-values were obtained from these analyses.

Table 1. Protocols used for T47D breast cancer cell and murine stroke brain tissue

Tissues			Spectrometer	Matrix	Mode	Results
T47D breast cancer cells						
Protocol 1	PVP 360 in 60% methanol (No change without trypsin)	Trypsin	Axima-CFR™*plus*	CHCA	Reflectron PSD	Histone H2A
Protocol 2	PVP 360 in 95% ethanol (No change without Trypsin)	Trypsin	Axima-CFR™*plus*	CHCA	Reflectron PSD	None
Protocol 3	Tween 20 in 60% methanol (No change without trypsin)	Trypsin	Axima-CFR™*plus*	CHCA	Reflectron PSD	None
Protocol 4	Tween 20 in 95% ethanol (No change without trypsin)	Trypsin	Axima-CFR™*plus*	CHCA	Reflectron PSD	None
Protocol 5	Cell lysis in distilled water (No change without trypsin)	Trypsin	Axima-CFR™*plus*	CHCA	Reflectron PSD	None
Murine stroke brain tissue		No trypsin	Axima-CFR™*plus*	Sinapinic	Linear MS	Serum albumin
		Trypsin	Axima-QIT™	DHB	Reflectron MS/MS	Tubulin β2 chain

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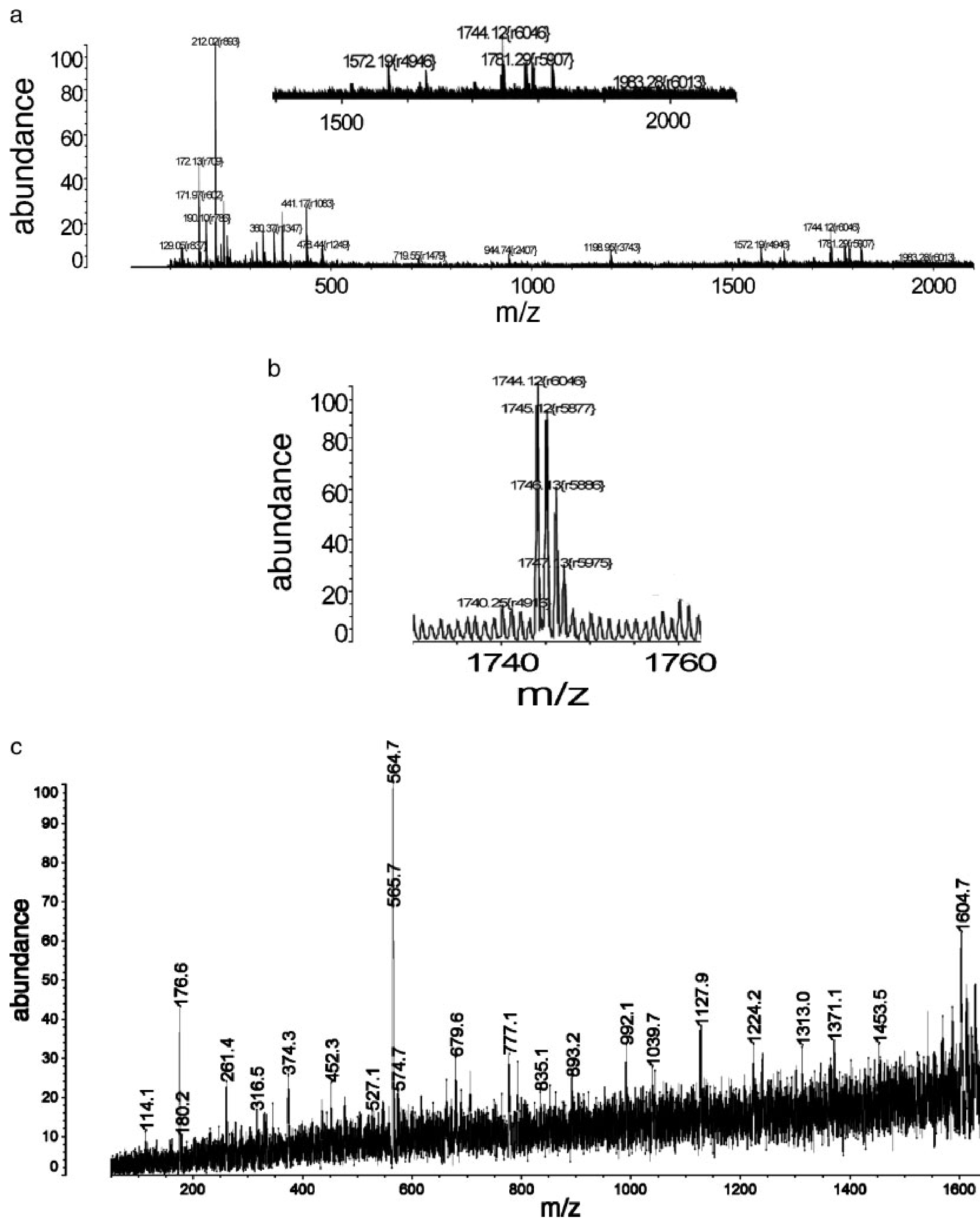

Figure 1. (a) T47D breast cancer cell MALDI MS spectrum demonstrating precursor ion AMGIMNSFVNDIFER $[M+H]^+$ at *m/z* 1742.8 (inset). (b) Demonstration of monoisotopic resolution of MALDI MS precursor ion AMGIMNSFVNDIFER $[M+H]^+$. (c) MALDI PSD product ion spectrum of ions from precursor ion AMGIMNSFVNDIFER $[M+H]^+$.

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Statistics

Since this was a feasibility study, no further statistics were applied except for the *p*-values obtained from each Mascot© search.

RESULTS

Performance of MALDI on pre- and post-trypsinized T47D breast cancer cells

The T47D cell breast cancer cells treated with 0.5% PVP 360 in 60% methanol, and then washed with 60% methanol to remove the PVP 360, gave the strongest MALDI signal of the five treatment protocols used in both the pre- and post-trypsinized cells. This treatment with PVP 360 in 60% methanol immediately fixed the cells and therefore cryoprotection was unnecessary because the cells were never frozen for preservation. Low signal intensity was obtained from three of the other four protocols, and no signal was obtained from the lysed cell protocol. The most abundant MALDI MS peptide ion was at *m/z* 1742.8. This ion was obtained from the cells treated with PVP 360 in 60% methanol and subsequently trypsinized. The spectra from the T47D breast cancer cells were virtually indistinguishable across the data sets from all 12 wells (N = 12) in both the MALDI MS and the MALDI PSD analyses. A MALDI spectrum, Figs. 1(a) and 1(b), and a MALDI PSD spectrum, Fig. 1(c), collected from a single well are shown as examples. The discrepancy between the initial precursor ion *m/z* value of 1743.1 on the PSD spectra compared with the *m/z* value of 1744.1 in the mass spectrum, and the difference between these two values, and the *m/z* value of 1742.8 on the Mascot© search are the result of different calibration files used on the same data.

Analysis of the MALDI PSD spectrum of the trypsinized T47D cells with Mascot© query of the NCBI database identified the protein histone H2B, Mascot© score 93, $p < 0.001$ (2e-07), molecular weight (MW) 13 942 Da, peptide mass tolerance: ±1.5 *m/z* units, fragment mass tolerance: ±1.5 *m/z* units, max. missed cleavages: 1. The detailed Mascot© search is presented in the Supplementary material (Supplementary Figures 1(a) and 1(b)).

Performance of MALDI on pre- and post-trypsinized non-stroke and stroke murine brain sections

Linear mode comparison of the non-trypsinized, left, non-lesioned (control) cerebral hemisphere, and non-trypsinized, right, lesioned cerebral hemisphere produced differential intensity (peak height) of a protein ion at an *m/z* value of 67k (Figs. 2(a) and 2(b)). The intensity of the ion at 67k was greatest at the center of the histologically confirmed stroke, no. 1 on the histopathology section (Fig. 3). The peak height decreased from the site of greatest brain injury in the stroke (no. 1 in Fig. 3) to the normal area in the ipsilateral (same side) brain cortex near the midline of the brain (no. 6, Fig. 3), and remained at this level throughout the entire left, non-stroke hemisphere. Two other protein ions at *m/z* values of 33k and 47k had almost the same intensities in both hemispheres (Figs. 2(a) and 2(b)), indicating that they may represent molecules not affected by the lesion. None of these proteins were identified. The spectra of normal and stroke brains were consistent across the data sets from all six animals (N = 6).

The most abundant MALDI MS peptide ion, LHFFMPGFAPLTSR $[M+H]^+$, from the trypsinized stroke brain was at *m/z* 1621.0. The spectra in all six animals were obtained from the largest area of the stroke (no. 1, Fig. 3) of the trypsinized mirror image right hemisphere stroke brain sections. The spectra from the stroke brains were virtually indistinguishable across the data sets from all six animals (N = 6). A MALDI spectrum and a MALDI CID spectrum from one animal are shown in Figs. 4(a), 4(b), and 4(c). Analysis of the CID spectrum of the trypsinized right hemisphere stroke brain with Mascot© query of the NCBI database matched the protein tubulin β2, Mascot© score of 47 and $p < 0.05$, MW 49 875 Da, peptide mass tolerance: ±0.5 *m/z* units, fragment mass tolerance: ±0.8 *m/z* units, and max. missed cleavages: 1. The detailed Mascot© search is presented in the Supplementary material (Supplementary Figure 2).

DISCUSSION

The purpose of this pilot study was to explore the feasibility of MALDI PSD/CID for *in situ* identification of proteins directly from fixed trypsinized T47D breast cancer cells and fixed trypsinized murine brain stroke tissue that had not undergone lysis, autolysis or desiccation.

Histone H2B in T47D breast cancer cells was identified by MALDI PSD of a precursor peptide ion, and tubulin β2 in murine stroke brain was identified by MALDI MS/MS (CID) of a precursor peptide ion. This study represents the first demonstration of histone H2B from T47D breast cancer cells and tubulin β2 from stroke brain.

Our technique differs from others in several ways.[3–15,22] The T47D breast cancer cells were fixed immediately with the solution of PVP 360 in 60% methanol, never frozen, and never lysed. The murine brain samples were cryoprotected with sucrose to prevent ice crystal formation upon instant freezing and interruption of metabolism by immersion in solidifying isopentane.[23] (Immersion directly into liquid nitrogen[24] was avoided because liquid nitrogen boils at −195.8°C, well below 37.7°C, the normal murine body temperature,[25] surrounds the tissue with an insulating layer of gas that prevents instant freezing, and permits continued metabolism such as protein phosphorylation.) The cryosections and cells were immediately immersed in 60% methanol to preserve cyto-architectural integrity and spatial relationships of the cut cells; tissue was transferred directly from the cryomicrotome stage to the metal plates and glass slides without intermediate transfer; 90 min trypsin digestion was chosen because it did not cause any apparent tissue alteration at the light microscopic level (data not shown), and assured adequate protein digestion;[26] and finally histone H2B and tubulin β2 were identified within 2 h in cells and tissues rather than after 13 h in non-cryoprotected, anatomically disrupted tissue as described by Shimma *et al.*[22]

Histone H2B, MW 13 942 Da, was identified from MALDI PSD analysis of T47D breast cancer cells. No definite ion at *m/z* 13 942 could be identified on the MALDI spectra, and therefore no correlation was observed between the MALDI spectra and the MALDI PSD spectra. Tubulin β2, MW

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Figure 2. (a) MALDI MS spectra collected in linear mode from a single animal from the non-stroke hemisphere. The individual spectra are color-coded and correspond to the color-coded sites of the origin of the spectra obtained from the section of stroke brain tissue (Fig. 3). (b) Linear mode MALDI MS spectra collected from the stroke hemisphere of a single animal. The relative abundance of the ions at *m/z* 67k (color-coded) varies with the size of the lesion. They range from lowest abundance in the normal non-lesioned hemisphere to highest at the largest size of the stroke (no. 1, Fig. 3). The color-coded spectra correspond to the colored numerals of the sampled loci in the photomicrograph (Fig. 3).

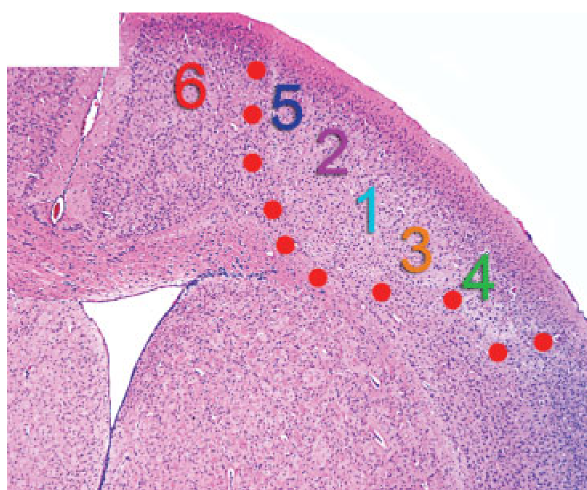

Figure 3. Example of a histological photomicrograph of the lesioned hemisphere (100×) from the same animal whose spectra are shown in Figs. 2(a) and 2(b). The periphery of the lesion is outlined by red dots to assist in visualization of the lesion. Note the edema (brain swelling indicated by the lighter appearance of the center of the lesion), hyperchromatic nuclei (dark appearing cells), and decreased number of neurons in the lesion compared with the surrounding normal brain beyond the periphery of the lesion. Linear mode MALDI MS sampling loci are marked by color-coded numerals corresponding to the observed spectra in Figs. 2(a) and 2(b). These numerals (1–5) represent the progressively decreasing lesion size, and normal cortical brain tissue near the midline (6).

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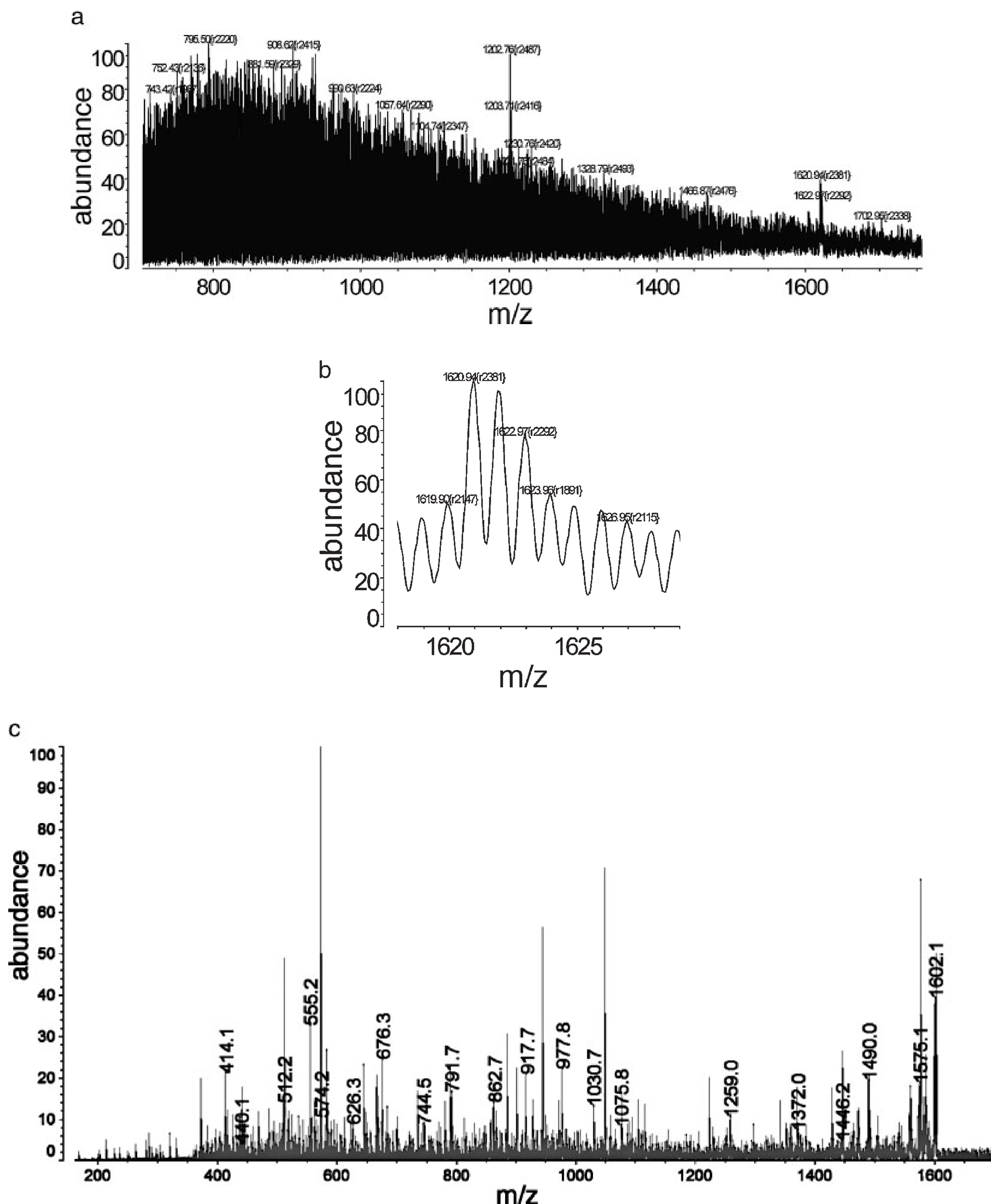

Figure 4. (a) Stroke brain tissue (trypsinized) MS spectrum demonstrating the precursor ion LHFFMPGFAPLTSR $[M+H]^+$ at *m/z* 1621.0. (b) Demonstration of monoisotopic resolution of the MALDI MS precursor ion LHFFMPGFAPLTSR $[M+H]^+$.

49 875 Da, was identified from the CID analysis. Since no ion at *m/z* 49 875 was identified in the non-trypsinized linear mode spectra of the stroke brains, no correlation was observed between these spectra and those of the post-trypsinized stroke brain CID spectra.

In our study of ischemic brain tissue (stroke), there was an apparent direct quantitative correlation between the spectra and the amount of edema present in the brain following the stroke. The ion at *m/z* 67k is likely to be mouse serum albumin. It is well known that serum albumin accumulates in the edematous brain at 24 h, and therefore this would be an expected finding.[27] The ion at *m/z* 33k could arise from a doubly charged 67k species, but only if their relative signal intensities rose in parallel in the non-stroke and stroke

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hemispheres. Since the intensity of the ion at *m/z* 33k remained the same in both hemispheres, the non-concordance of intensities of the ions at *m/z* 33k and 67k between the normal and stroke hemispheres suggests that they are unlikely to be singly and doubly charged molecules of the same species.

We used a chemical printer to spot trypsin and matrix accurately on the T47D cells and stroke brain tissue, and transferred this raster to the mass spectrometer. Chemical printers were first described by Adams in 1984, later by both Spengler and Sloane in 2002, and more recently by Aerni.[28–31] We chose the technique described by Sloane *et al*.[30] because it provides microscopic visualization of the tissue loci, real-time observation of the droplet application to the tissue surface, and co-registration that permits precise robotic control for laser exposure in the MALDI instrument.

Embedding media for cryosection, such as agar, a polysaccharide, and OCT (Sakura Finetek, Torrance, CA, USA), a combination of polyvinyl alcohol and polyethylene glycol polymers, were avoided as they suppress ion formation in MALDI MS.[24,32]

This report demonstrates that MALDI PSD and MALDI CID can furnish rapid, direct identification of proteins *in situ* from cells and tissue. In this preliminary study only a single protein, histone H2B, was identified from the breast cancer cells, and similarly a single protein, tubulin β2, was identified from the stroke portion of the murine brains. While only one precursor peptide was used to match the proteins from the Mascot searches, the search parameters of $\pm$1.5 *m/z* units or less for the precursor and fragment peptide ions for histone H2B, and $\pm$0.5 and $\pm$0.8 for the precursor and fragment peptide ions for tubulin β2, with the Mascot scores of 93 and 47, respectively, for histone H2B and tubulin β2, the low *p*-values of $<$0.001 (2e-07) and $<$0.05, respectively, for histone H2B and tubulin β2, and the high number of y-series matches strongly validate the results obtained in identifying histone H2B from breast cancer cells and tubulin β2 from stroke brain tissue. Western blots or ELISA techniques would require extraction of protein from the cells and tissue, and would not be consistent with identification of proteins directly from cells and tissue. In future experiments, the focus will be on the refinement of the tissue preparation to increase detection of low abundance proteins in cells and tissue.

CONCLUSIONS

This preliminary study demonstrates that direct protein identification from cells and tissue by MALDI PSD/CID can be performed quickly and easily and may become the basis for identification of protein and non-protein molecular biomarkers of clinical disease. Direct tissue MALDI could potentially move from the laboratory to clinical practice because of the relative ease and speed of tissue preparation.

SUPPLEMENTARY MATERIAL

The supplementary electronic material for this paper is available in Wiley InterScience at: http://www.interscience.wiley.com/jpages/0951-4198/suppmat/

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