Pressure BioSciences Inc.

PCT NEWS

OTCQB: PBIO
Discovery Starts With Sample Preparation™

Volume 8, Issue 6 June 2014

Transformative Impact and Adoption of Pressure BioSciences' PCT Platform Prominently Highlighted Throughout Week at Major Scientific Conference

PCT-based Sample Preparation Methods and Enabling Impact Featured in Eight Presentations Over Four Days in Cancer, Tissue Biopsy, Archival Samples, and Food-borne Pathogen Studies

Transformative Impact and Adoption of Pressure BioSciences' PCT Platform Prominently Highlighted Throughout Week at Major Scientific Conference

PCT-based Sample Preparation Methods and Enabling Impact Featured in Eight Presentations Over Four Days in Cancer, Tissue Biopsy, Archival Samples, and Food-borne Pathogen Studies

South Easton, MA, June 19, 2014 – Pressure BioSciences, Inc. (OTCQB: PBIO) ("PBI" or the "Company") today announced that scientists from six separate research groups presented data at the 62nd Annual Conference of the American Society for Mass Spectrometry ("ASMS") being held from June 15-19, 2014 in Baltimore, MD. Study results indicated that utilizing the Company's patented pressure cycling technology ("PCT") platform in the preparation of samples for analysis resulted in critically enabling quality and/or improved time or cost efficiency of test results. These studies were conducted by scientists from ETH Zurich and the University of Zurich ("ETH Zurich"); Laboratory Corporation of America ("Lab Corp"); the Food and Drug Administration ("FDA"); the University of Minnesota ("UMN"), Mayo Clinic, and the Karolinska Institute; Northeastern University ("NEU"); and the Baltimore VA Medical Center ("BVAMC"), SAIC-Frederick, National Cancer Institute, and the Veteran's Health Administration.

ETH Zurich. Mass spectrometry is an important method for the characterization of proteins (and other biomolecules). Dr. Tiannan Guo (laboratory of Dr. Ruedi Aebersold, internationally acclaimed protein chemist and Professor of Molecular Systems Biology at ETH Zurich and the University of Zurich), presented on both Sunday June 15th and Wednesday June 18th on PCT SWATH, a method they developed that combines the many advantages of PCT with SWATH, a revolutionary mass spectrometry method from AB SCIEX. Dr. Guo's and Professor Aebersold's results indicate that PCT SWATH could significantly reduce overall processing times for protein analysis and biomarker discovery by mass spectrometry while concomitantly decreasing test cost and increasing test quality. AB SCIEX is a global leader in life science and analytical technologies, including mass spectrometry.

Continued on Page 2, Column 1

UPCOMING EVENTS

Scientific Meeting: PBI to showcase the Company's PCT-based systems and new products for sample preparation as well as Constant Systems' cell disruption equipment at 28th Annual Protein Society Symposium, July 27 - 30, 2014 at the Manchester Grand Hyatt in San Diego, California

Excerpts from PCT-related Poster Abstracts from ASMS 2014

A rapid, data independent acquisition method for population-scale proteome barcoding using PCT-SWAT

Tiannan Guo; Ruedi Aebersold; *ETH Zurich, Zurich, Switzerland*

Introduction

Population-scale proteomic analysis is essential for biomarker studies and, more broadly, for personalized and precision medicine. A barcode is the representation of data that can be used to rapidly identifying a unique object. Biological samples from different species are being catalogued using DNA barcodes, however, DNA is not the ideal material to distinguish samples from the same species. We introduce here the concept "proteome barcoding" as a new mass spectrometry based methodology for producing representative proteomic BGI DATA for population-scale biological samples. The methodology has 3 key features: 1) fast digitization of proteome; 2) minimal sample consumption and, 3) reproducible and comprehensive BIG proteomic DATA.

Novel Aspect

Here we move proteomics to population-scale analysis. We have coined a new concept "proteome barcoding"

Variables Affecting the Quality of Digestion-based Protein Quantification: Implications of Enzyme Kinetics on Clinical Measurements

Christopher Shuford; Martin Green; Russell Grant; *Laboratory Corporation of America, Burlington, NC*

Introduction

It has recently been demonstrated rapid proteolysis is critical to achieving accurate quantitation in protein cleavage isotope-dilution mass spectrometry (PC-IDMS) assays when the surrogate/target peptide is labile. Given the inherent implication to clinical measurements, we have performed kinetic studies of trypsin-based proteolysis for 8 potential surrogate peptides of Thyroglobulin, a tumor marker which is often quantified by PC-IDMS in clinical settings due to interference in immunometric measurements. Multiple factors purported to increase the rate of proteolysis were evaluated to determine their impact on quantitative accuracy, including the influence of competitive interference on the rate of proteolysis. Cleavable stable isotope-labeled (SIL) peptides were tested for their ability to correct for variance in the rate and efficiency of proteolysis across various conditions.

Novel Aspect

These studies demonstrate utilizing stable surrogate peptides and increasing rates of proteolysis are paramount for achieving absolute accuracy by PC-IDMS.

Click on Any Abstract Title for the Complete Version

Continued on Page 2, Column 2

PBI Pressure BioSciences Inc.

PCT NEWS

OTCQB: PBIO

Discovery Starts With Sample Preparation™

Volume 8, Issue 6 June 2014

Transformative Impact and Adoption of Pressure BioSciences' PCT Platform Prominently Highlighted Throughout Week at Major Scientific Conference: Continued from Page 1, Column 1

Lab Corp. Thyroid cancer accounts for approximately 4% of all new cancers each year in the United States. Deaths are uncommon if detected early as it can usually be treated and cured once diagnosed. Dr. Christopher Shuford and colleagues presented data on the development of a mass spectrometry-based thyroid cancer test. A major step in the potential test workflow is the rapid digestion of the thyroglobulin protein and its subsequent identification by mass spectrometry. PCT was shown to digest thyroglobulin significantly faster than all three comparative methods, including two microwave systems (current competitive alternatives to PCT for protein digestion) and the Lab Corp standard heat-based method.

FDA. There are more than 250 different food-borne diseases. They are caused by bacteria, other pathogens, and toxins. Symptoms range from mild disease to life-threatening conditions. Dr. Melinda McFarland and colleagues presented data on the development of a mass spectrometry-based method to improve the identification of bacteria involved in food-borne outbreaks by testing contaminated food samples. Thirty-six Salmonella isolates originating from food-borne outbreaks were studied. PCT was the method of choice for extracting the pathogen from the samples. Current methods to determine the causative agent of food-borne outbreaks primarily use DNA detection. The authors showed that bacterial protein expression profiles could potentially enhance pathogen identification in food-borne outbreaks.

UMN and Others. COPD is a leading cause of death in the United States. It is a serious lung condition and a key risk factor for lung cancer. There is no cure and no way to reverse the damage done by the condition. The goal of the study was to find novel biomarkers in COPD-associated lung cancer tissue samples and to eventually apply the detection of these biomarkers to non-invasively collected bronchoalveolar lavage. In their presentation, Dr. Brian Sandri and colleagues reported on the discovery of target pathways and promising biomarkers of COPD-associated lung cancer. PCT was the sample preparation method of choice, as the authors had previously shown that PCT could extract substantially more proteins from lung tissue than other extraction methods.

NEU and Others. Peganum harmala (P. harmala) is a perennial plant, also known as Syrian Rue. The seed has been used for medicinal purposes and as a condiment. The seed contains the hallucinogenic and narcotic compounds harmine and harmaline. The purpose of the study was to evaluate DMS mass spectrometry as a potentially better analysis method for the characterization of suspected P. harmala seeds, as compared to GC mass spectrometry. PCT was the method of choice for sample preparation, for as Dr. Adam Hall and colleagues stated: "to illustrate a faster and more efficient extraction method, harmine and harmaline were extracted from seeds using an adapted method based on PCT". The authors reported that PCT followed by the rapid separation and analysis by DMS mass spectrometry enhanced the detection of harmine and harmaline, as compared to standard GC mass spectrometry.

Continued on Page 3, Column 1

Excerpts from PCT-related Poster Abstracts from ASMS 2014: Continued from Page 1, Column 2

Serovar and strain level bacterial differentiation capabilities for 36 closely related outbreak strains by intact protein LCMS

Melinda McFarland; Denis Andrzejewski; Peter Evans; John Callahan; *US Food & Drug Administration, College Park, MD*

Introduction

ESI-LCMS analysis of intact protein bacterial lysates generates unique protein expression profiles for bacterial differentiation. For traceback of food contamination serovar level differentiation is necessary and strain level typing ideal. Most bacterial protein LCMS efforts have been limited to comparison between two samples. We present LCMS intact protein expression profile results from participation in a semi-blinded study of 36 Salmonella isolates originating from foodborne outbreaks. Study creators established sample relatedness at the serotype, PFGE, and whole genome sequence levels. LCMS generated intact protein expression profiles were used to divide isolates into resolvable clades. These clades were compared to those generated from the above mentioned genomic techniques.

Novel Aspects

Serovar and strain level bacterial differentiation capabilities of intact protein LCMS for 36 closely related Salmonella outbreak strains.

Large-Scale Quantitative Proteomic/Metaproteomic Platform Discovers Target Pathways and Promising Biomarkers of COPD-associated Lung Cancer

Brian Sandri[1]; Andy Limper[2]; Pratik Jagtap[4]; Ping Yang[2]; Ola Larsson[3]; Peter Bitterman[1]; Tim Griffin[4]; Leeann Higgins[4]; Todd Markowski[4]; Chris Wendt[1]; *[1]University of Minnesota, Minneapolis, MN;[2]Mayo Clinic, Rochester, MN; [3]Karolinska Institutet, Solna, Sweden; [4]Mass Spectrometry and Proteomics, UMN, Minneapolis, MN*

Introduction

Chronic Obstructive Pulmonary Disease (COPD), independent of smoking, is an emerging risk factor for lung cancer. Unfortunately, little has been accomplished to understand this link, and we lack data on potential therapeutic targets and biomarkers indicative of COPD-associated lung cancer. Furthermore, although appreciated, we lack understanding of the contribution of the lung microbiome on these conditions. To fill this gap, we have developed a comprehensive analysis platform employing innovative clinical sample preparation, iTRAQ labeling, and computational analysis using the flexible Galaxy-P platform. This platform enables comprehensive quantitative proteomic/metaproteomic analysis in tens of patient samples. We have generated novel data on disease-associated host proteins and microbiome contributions, identifying affected molecular pathways and promising biomarkers of COPD-associated lung cancer.

Novel Aspects

A unique platform enabling comprehensive identification of molecular pathways, biomarkers, and microbiome contributed proteins to COPD-associated lung cancer

Click on Any Abstract Title for the Complete Version

Continued on Page 3, Column 2

PBI Pressure BioSciences Inc.

PCT NEWS

OTCQB: PBIO
Discovery Starts With Sample Preparation™

Volume 8, Issue 6 June 2014

Transformative Impact and Adoption of Pressure BioSciences' PCT Platform Prominently Highlighted Throughout Week at Major Scientific Conference: Continued from Page 2, Column 1

BVAMC and Others. Formalin fixation followed by paraffin embedding ("FFPE") is the most commonly used method worldwide for the preservation of tissues for pathology evaluation. Archival repositories that contain millions upon millions of FFPE tissue samples represent an invaluable resource for retrospective studies of disease progression and response to therapy. Unfortunately, the analysis of FFPE samples is highly problematic because molecules (including proteins) of interest are chemically trapped in the tissue samples by formalin fixation. Nonetheless, in two different presentations, Dr. Carol Fowler, Dr. Jeffrey Mason, and colleagues reported substantial improvements in protein recovery from FFPE tissue samples of four-fold (4x) when extraction was performed by PCT.

Dr. Nate Lawrence, Vice President of Marketing and Sales, said: "We are having a very successful ASMS Meeting. The launch of our new high throughput Barozyme HT48 has gone better than expected, with strong interest reflected in our booth traffic and meetings. The PCT platform has also received a lot of publicity with eight presentations by six separate research groups on the uses and advantages of PCT."

Dr. Lawrence continued: "We are pleased and very encouraged with the presentations on PCT by many prominent scientific clients over the past four days. We believe the data they reported support the use of PCT in a number of new, exciting, and potentially financially rewarding areas, and that their data will encourage and accelerate new researchers to try PCT. In that regard, we are particularly pleased with the results reported by Dr. Guo and Dr. Aebersold on the advantages of PCT SWATH, as their workflow utilized our new patent-pending PCT micro-Pestle. We believe that the advantages of this new device for the extraction of protein from very small biopsy samples go well beyond current competitive products, and that the PCT micro-Pestle will begin to positively affect revenue as early as Q3 2014".

For more information about PBI and this press release, please click on the following website link:

http://www.pressurebiosciences.com

About Pressure BioSciences, Inc.
Pressure BioSciences, Inc. ("PBI") (OTCQB: PBIO) develops, markets, and sells proprietary laboratory instrumentation and associated consumables to the estimated $6 billion life sciences sample preparation market. Our products are based on the unique properties of both constant (i.e., static) and alternating (i.e., pressure cycling technology, or PCT) hydrostatic pressure. PCT is a patented enabling technology platform that uses alternating cycles of hydrostatic pressure between ambient and ultra-high levels to safely and reproducibly control bio-molecular interactions. To date, we have installed over 250 PCT systems in approximately 160 sites worldwide. There are over 100 publications citing the advantages of the PCT platform over competitive methods, many from key opinion leaders. Our primary application development and sales efforts are in the biomarker discovery and forensics areas. Customers also use our products in other areas, such as drug discovery & design, bio-therapeutics characterization, soil & plant biology, vaccine development, histology, and counter-bioterror applications.

Forward Looking Statements
Statements contained in this press release regarding PBI's intentions, hopes, beliefs, expectations, or predictions of the future are "forward-looking" statements within the meaning of the Private Securities Litigation Reform Act of 1995. These statements are based upon the Company's current expectations, forecasts, and assumptions that are subject to risks, uncertainties, and other factors that could cause actual outcomes and results to differ materially from those indicated by these forward-looking statements. These risks, uncertainties, and other factors include, but are not limited to, the risks and uncertainties discussed under the heading "Risk Factors" in the Company's Annual Report on Form 10-K for the year ended December 31, 2013, and other reports filed by the Company from time to time with the SEC. The Company undertakes no obligation to update any of the information included in this release, except as otherwise required by law.

Excerpts from PCT-related Poster Abstracts from ASMS 2014: Continued from Page 2, Column 2

Rapid Identification of Beta-carboline Hallucinogens: Harmine and Harmaline, by Pressure Cycling Technology (PCT) and DMS-MS

Adam B. Hall[1]; Amol Kafle[1]; Alex Thompson[3]; Frederick Li[2]; Kaitlyn Duffy[1]; James Glick[1]; Stephen L. Coy[1]; Paul Vouros[1]; *[1]Northeastern University, Boston, MA; [2]Boston University School of Medicine, Boston, MA; [3]Vermont Forensic Laboratory, Waterbury, VT*

Introduction
A comparative analysis was performed using GC/MS and Differential Mobility Spectrometry - Mass Spectrometry (DMS-MS) for the analysis of Peganum harmala seeds. Ions corresponding to the ?-carboline hallucinogens, harmine and harmaline, which differ by one level of saturation in their pyridine rings, were separated and detected from a single seed prepared for analysis utilizing pressure cycling technology. A direct comparison between GC/MS and DMS-MS is shown in an effort to evaluate DMS as a rapid analysis method for trace drugs of abuse from plant-based matrices. DMS prior to mass analysis allows an analyst to separate a population of electrosprayed ions and has demonstrated promising research findings for the high throughput analysis of forensically relevant and structurally similar drugs of abuse.
Novel Aspects
Enhanced sensitivity by PCT followed by rapid separation and analysis by DMS-MS for structurally similar Beta-carboline hallucinogens: harmine and Harmaline.

High Pressure-Assisted Extraction for the Improved Proteomic Analysis of FFPE Tissue

Carol B. Fowler[1]; Timothy J. Waybright[2]; Timothy D. Veenstra[2]; Timothy J. O'Leary[3]; Jeffrey T. Mason[1]; *[1]Baltimore VA Medical Center, Baltimore, MD; [2]National Cancer Institute, Frederick, MD; [3]BLR&D Service, Veterans Health Administration, Washington, DC*

Introduction
Mass Spec-based proteomics hold great promise for developing knowledge of the molecular characteristics of disease. Formaldehyde-fixed, paraffin-embedded (FFPE) tissue repositories represent an invaluable resource for the retrospective study of disease progression and response to therapy. However, the analysis of FFPE tissues by proteomic methods has been hampered by formaldehyde-induced protein adducts and cross-links. Here, we demonstrate the use of heat augmented with high hydrostatic pressure (40,000 psi) as a novel method for the recovery of intact proteins from FFPE mouse liver.
Novel Aspects
This study shows that high pressure treatment improves the quality and yield of proteins extracted from archival tissue.

Click on Any Abstract Title for the Complete Version

Continued on Page 4, Column 1

PBI Pressure BioSciences Inc.

PCT NEWS

OTCQB: PBIO

Discovery Starts With Sample Preparation™

Volume 8, Issue 6 June 2014

Excerpts from PCT-related Poster Abstracts from ASMS 2014:
Continued from Page 3, Column 2

Elevated Pressure Improves the Extraction and Identification of Proteins Recovered from Formalin-Fixed, Paraffin-Embedded Tissue Surrogates

Carol Fowler[1]; Cedric Moore[3]; Timothy O'Leary[2]; Jeffrey Mason[1]; *[1]Baltimore VA Medical Center, Baltimore, MD; [2]Veterans Health Administration, Washington, DC; [3]Johns Hopkins University, Baltimore, MD*

Introduction

Proteomic studies of formalin-fixed paraffin-embedded (FFPE) tissues have been hampered by the inability to extract proteins from archival tissue in a form suitable for analysis by 2-D gel electrophoresis or mass spectrometry. This inability arises from the difficulty of reversing formaldehyde-induced protein adducts and cross-links within FFPE tissues. We previously reported the use of elevated hydrostatic pressure as a method for efficient protein recovery from a hen egg-white lysozyme tissue surrogate, a model system developed to study formalin fixation and histochemical processing.

Novel Aspects

These mechanistic studies demonstrate that elevated pressure treatment is a promising approach for improving proteomic analysis of FFPE tissue.

Alternation of Glycans Site Specificity in Patients with Liver Diseases

Petr Pompach[1, 2]; Petra Darebna[2]; Petr Novak[1, 2]; Ondrej Topolcan[3]; Julius Benicky[4]; Miloslav Sanda[4]; David Ashline[5]; Radoslav Goldman[4]; *[1]Institute of Microbiology ASCR, Prague, Czech Republic; [2]Faculty of Science, Charles University, Prague, Czech Republic; [3]Faculty Hospital in Pilsen, Pilsen, Czech Republic;[4]Georgetown University, Washington, DC, DC; [5]University of New Hampshire, Durham, NH*

Introduction

Glycosylation dramatically influence biochemical properties of proteins. It is known that glycans structure changes under non-physiological conditions such as disease or inflammation. These changes have diagnostic potential and could be used as disease biomarkers. The maturation of glycans is controlled not only by the enzyme activities of glycosidases and glycosyltransferases, but also by the protein structure. Here we present data showing the site specific glycan alternation of several serum proteins isolated from patients with liver diseases.

Novel Aspects

Glycan site specificity is protein and peptide specific.

Click on Any Abstract Title for the Complete Version

Continued on Page 4, Column 2

Excerpts from PCT-related Poster Abstracts from ASMS 2014:
Continued from Page 4, Column 1

Mass spectrometry-based proteomics of human induced pluripotent stem cells (hiPSC) cultured in suboptimal culture conditions

Melkamu Getie-Kebtie[1]; Natalia Pripuzova[1]; Christopher Grunseich[2]; Colin Sweeney[3]; Harry Malech[3]; Michail Alterman[1]; *[1]Division of Cell and Gene Therapy, CBER, FDA, Bethesda, MD; [2]Neurogenetics Branch, NINDS, NIH, Bethesda, MD; [3]Laboratory of Host Defenses, NIAID, NIH, Bethesda, MD*

Introduction

Human induced pluripotent stem cells (hiPSCs) offer unprecedented potential for drug discovery, toxicology, regenerative medicine, and disease research. The quality of the stem cells has a great impact on how the cells could be utilized in future applications. Suboptimal culture conditions, such as prolonged culturing beyond confluency may result in metabolic and phenotypic changes in cells that may pose safety risks or produce inferior quality of products. Currently there is no effective technique available to monitor global quality of hiPSC in cell culture. Here we applied comprehensive qualitative and quantitative proteomics to monitor proteome changes during the course of prolonged culture (suboptimal culture conditions) and aimed to identify a panel of proteins that could predict the quality of cells.

Novel Aspects

Identification of a panel of proteins that could potentially be used for prediction of cell quality