

Pressure BioSciences, Inc. Awarded NIH SBIR Phase I Grant for the Development of an Automated, Pressure-Enhanced System for the Routine Processing of Cancer and Other Tissue Samples

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South Easton, MA, September 6, 2011 – Pressure BioSciences, Inc. (NASDAQ: PBI) (“PBI” and the “Company”) today announced that it has been awarded a \$160,978 SBIR Phase I grant (1R43GM090582-01A1) from the National Institute of General Medical Sciences (“NIGMS”) of the National Institutes of Health (“NIH”). Entitled “Methods and Instrumentation for Hydrostatic Pressure-Enhanced Tissue Fixation”, the grant will help fund the development of a high pressure system for the automated processing and preservation of tissue samples to facilitate, standardize, and improve the tissue fixation process independent of sample size and tissue type. If the Phase I development efforts are successful, the Company expects to submit a request for SBIR Phase II funding; such funding is usually for approximately \$1 million and is billed over a two year period.

As announced in May of 2010, the Company has a Cooperative Research and Development Agreement (“CRADA”) with the Armed Forces Institute of Pathology (“AFIP”), the American Registry of Pathology (“ARP”), and the Department of Veterans Affairs (“VA”). One of the purposes of the CRADA is to develop pressure-based methods to improve the quality and speed of preparing formalin fixed, paraffin embedded (“FFPE”) tissues. AFIP will provide PBI with laboratory analyses and histology services under the grant.

Formalin fixation followed by paraffin embedding is the most commonly used technique worldwide for the preservation of tissues for pathology evaluation. However, the quality and analysis of FFPE samples are highly problematic for a number of reasons, including a lack of standardization in both the times and methods currently used for tissue fixation, and the inconsistent penetration of formalin in various tissue types. Inconsistent formalin penetration can lead to uneven fixation and a subsequent “corruption” of the tissue sample.

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**Scientists from Florida International University (FIU) and Virginia Commonwealth University will Present Their Research Using Pressure Cycling Technology (PCT) for Studies in Sexual Assault Cases and Increased Yield of Low Copy DNA from Forensic Samples at the
22nd International Symposium On Human Identification (ISHI)
National Harbor, MD October 3–6, 2011**

22 Application of Pressure Cycling Technology (PCT) in Differential Extraction

Deepthi Nori*, MFS; Bruce R. McCord, Ph.D., Florida International University, Department of Chemistry and Biochemistry, 11200 SW 8th St, Miami, FL 33199

Forensic DNA analysis has elevated the degree of confidence in the analysis and interpretation of evidence but the bottle neck that plagues the crime labs across the country is the tedious, time consuming protocols that require practice and expertise in analyzing mixtures. Organic differential extraction is the most commonly used method to isolate sperm DNA from sexual assault evidence. This two-step extraction procedure involves selective digestion of epithelial cells in the first step followed by isolation and digestion of sperm cell pellet. The major disadvantages of this technique are incomplete separation of sperm and non-sperm fractions, particularly in samples that are overwhelmed by large numbers of female epithelial cells relative to sperm cells and time-consuming nature of the process.

Pressure cycling technology sample preparation system (PCT SPS) involves the use of pressure pulses to disrupt tissues, cells and cellular structures enabling the recovery of their

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PBI EVENTS

PBI to showcase pressure cycling technology (PCT) and its applications in forensics at the 22nd International Symposium on Human Identification (ISHI) in National Harbor, MD. October 3–6, 2011. Posters by Deepthi Nori (FIU), Jessica Barker (Virginia Commonwealth University) and Julian Mendel (FIU) will be presented showing their work using PCT for studies in sexual assault cases and increased yield of low copy DNA from forensic samples.

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Dr. Jeffrey T. Mason, Chairman of the Biophysics Department at AFIP, commented: "Formalin-based tissue histology has remained largely unchanged for nearly half a century. The slow rate of penetration of formalin into tissues results in uneven fixation and increases the time to diagnosis. Preliminary studies indicate that pressure-assisted histology has the potential to significantly decrease the time required for tissue processing and subsequent examination by a pathologist. Further, pressure-assisted histology results in more uniform chemical and antibody staining, both of which improve the ability of the pathologist to evaluate the cellular architecture of the tissue."

Dr. Alexander V. Lazarev, Vice President of R&D for PBI, said: "We believe that there are over 35 million new FFPE tissue samples processed each year in the U.S. alone. In addition to their importance in pathology evaluation, an increasing number of these samples are being used for biomarker discovery, where the quality of fixation is crucial for the preservation of molecules vital to the understanding of disease mechanisms and drug development. We believe our pressure-enhanced fixation method can significantly increase the quality of the standard fixation process. Such improvement could lead to the generation of better data from FFPE samples, which could then lead to faster disease diagnosis, as well as the successful development of new diagnostics, therapeutics, and vaccines."

About Pressure BioSciences, Inc.

Pressure BioSciences, Inc. (PBI) is a NASDAQ Capital Market listed company focused on the development and sale of instrumentation and consumables based on a novel, enabling technology platform called Pressure Cycling Technology (PCT). PCT uses cycles of hydrostatic pressure between ambient and ultra-high levels (up to 35,000 psi and greater) to control bio-molecular interactions. PBI currently holds 24 issued patents covering multiple applications of PCT in the life sciences field, including genomic and proteomic sample preparation, pathogen inactivation, the control of chemical reactions, immunodiagnosics, and protein purification. PBI currently focuses its efforts on the development and sale of PCT-enhanced bio-molecule extraction and enzymatic digestion products designed specifically for the mass spectrometry marketplace, as well as sample preparation products for biomarker discovery, soil and plant biology, forensics, histology, and counter-bioterror applications

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components. Barocycler® NEP2320, a commercially available instrument from Pressure Biosciences Inc, is equipped with a hydrostatic pressure chamber that generates alternating cycles of ambient and high pressure up to 45000 psi resulting in the lysis of cells.

The current study involves the application of pressure cycling technology in the selective digestion of sperm cells from evidence mixtures collected from different substrates with an emphasis on the role of buffer composition on sperm DNA yields. The cells were extracted into 1X PBS buffer (pH 7.4) with varying buffer compositions and subjected to 45000 psi pressure for 60 cycles. Samples were placed in specially designed PULSE™ tubes and introduced into the pressure chamber. This pressure treatment was followed by phenol chloroform isoamyl alcohol purification to obtain a clean DNA sample devoid of salts and proteins for successful downstream analysis. The purified DNA was quantified with Promega Plexor® HY system.

According to our previous studies, high selectivity and improved recovery with the reducing agent, Tris (2-carboxyethyl) phosphine (TCEP) indicated the potential for highly selective detection of sperm cells in comparison to the addition of detergents or changes in temperature. These observations were applied to mixture studies of evidence obtained from various substrates such as swab and fabric. Preliminary data indicates that pressure cycling technology has application in differential extractions indicating improved extraction of sperm DNA at high pressures when compared to epithelial cells in the presence of appropriate buffers.

24 THE UTILIZATION OF PRESSURE CYCLING TECHNOLOGY TO ENHANCE DNA EXTRACTION FROM LOW TEMPLATE SAMPLES

Jessica Barker, M.S.*¹, Susan A. Greenspoon, Ph.D.², and Brad Jenkins, M.S.²

1. Virginia Commonwealth University, Richmond, VA
2. Virginia Department of Forensic Science, Richmond, VA

Low template DNA analysis has become a primary focus of forensic laboratories as scientists attempt to develop DNA profiles from increasingly smaller quantities of DNA and/or deposited on challenging substrates. Low template analysis techniques have been developed to increase sensitivity and elucidate profiles from these low level samples. However, most of these methods involve post-extraction enhancements which currently prevent the resulting profiles from being uploaded to NDIS.

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24 THE UTILIZATION OF PRESSURE CYCLING TECHNOLOGY TO ENHANCE DNA EXTRACTION FROM LOW TEMPLATE SAMPLES: Continued from Page 2/Column 2

Allelic peak imbalance, drop-out, drop-in, and increased stutter peaks are commonly observed when low template DNA is STR amplified and analyzed. The development of techniques that increase DNA extraction efficiencies from low template samples may eliminate the necessity for post-extraction procedures to obtain meaningful profiles from low level samples. Pressure cycling technology (PCT), the application of rapid cycles of ultrahigh hydrostatic and ambient pressure to biomolecular samples, was evaluated as a method to enhance DNA extraction from low template samples when used in conjunction with current extraction techniques. The extraction procedure utilized for these experiments was a Proteinase K digestion followed by purification using the DNA IQ™ System on the Biomek® NX^P automation platform. DNA was extracted from swabs containing neat blood diluted to low template levels (approximately 100 pg). Average DNA quantitation values obtained from PCT-treated samples were compared to control sample values and evaluated for statistical significance ($\alpha=0.05$). Treatment of swabs with PCT prior to incubation and purification increased DNA yields, whereas post-incubation treatment did not. Average DNA concentrations of low template samples increased up to 58% when using a Proteinase K digestion buffer containing Sarkosyl and increased up to 33% when an SDS containing digestion buffer was utilized. When only DNA IQ™ Lysis buffer was used for both extraction and purification, PCT treatment didn't affect DNA yields. Currently, no explanation is available for this observation. STR profiles and typing performance including peak heights and allelic drop-out rates were not adversely affected when pressure cycling technology was employed. STR typing results and peak heights correlated to DNA concentrations produced when using the various buffer configurations for both the controls and PCT-treated samples, except for some samples processed using the SDS containing Proteinase K buffer. PCR inhibition due to residual SDS in the DNA extract is the suspected cause. Average peak heights increased up to 35% when the Sarkosyl-containing buffer was utilized. However, DNA IQ™ Lysis buffer without PCT treatment yielded the highest average DNA concentrations and STR peak heights.

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27 EVALUATION OF A NOVEL DNA EXTRACTION TECHNIQUE, FOR OPTIMAL RECOVERY OF DNA FROM EPITHELIAL CELLS

Julian Mendel¹ and DeEtta Mills² PhD
¹International Forensic Research Institute
Department of Chemistry and Biochemistry
²Department of Biological Sciences
Florida International University

In the field of forensic science, there is a constant demand for new and improved techniques to enhance the information one can gather from the biological trace evidence. Humans shed approximately 400,000 epithelial cells daily which can be a useful source of DNA evidence. However, DNA obtained from touch samples, that leave only a few cells behind can prove to be difficult as the collection and the extraction process result in significant loss of valuable DNA. It is therefore essential that the procedures from sample collection to DNA extraction to PCR and genetic profiling be optimized to ensure the best possible results from low abundance samples. This study is a comparative analysis between commercially available Qiagen DNA extraction kits and the novel extraction technique, pressure cycling. Pressure Cycling Technology (PCT) uses rapid cycles of hydrostatic pressure ranging from ambient to ultra-high levels to disrupt bio-molecular interactions. Cultured, keratinocytes were re-suspended in basal growth media, counted using a hemocytometer and approximately 10,000 cells were placed into tubes as well as spotted directly onto 1.2µm polycarbonate filters and the Qiagen MicroKit DNA extraction protocol was performed. For comparison 10,000 cells was placed directly into the pressure cycling tubes as well as onto filters, the filters then placed into pressure cycling tubes and 1.5 mL buffer ATL (Qiagen) and 20µL of Proteinase K (>600 mAU/mL) were added. These samples were subjected to 20 cycles of 0 to 35000 PSI using the Barocycler NEP2320. Following the initial PCT step, samples were subjected to the Qiagen column clean up per manufacturers' protocols. The two methods were compared using quantitative PCR and the addition of pressure cycling to the standard Qiagen protocols resulted in an increase in DNA yield of 16%, when pressure cycling is used vs. Qiagen alone. Further experiments with dilution series of cells ranging from 10,000 to 50 cells as well as touch samples collected on porous and non-porous surfaces is currently being tested.

[Excerpt from the Journal of Cancer 2010;1:178-183](#)

Elevated Pressure Improves the Rate of Formalin Penetration while Preserving Tissue Morphology

Ingrid E. Chesnick¹, Jeffrey T. Mason¹, Timothy J. O'Leary², Carol B. Fowler^{1,2} □

1. Department of Biophysics, Armed Forces Institute of Pathology, Rockville, Maryland, USA;

2. Biomedical Laboratory Research and Development Service, Veterans Health Administration, Washington, DC, USA

Abstract

Formaldehyde fixation and paraffin-embedding remains the most widely used technique for processing cancer tissue specimens for pathologic examination, the study of tissue morphology, and archival preservation. However, formaldehyde penetration and fixation is a slow process, requiring a minimum of 15 hr for routine processing of pathology samples. Routinely fixed samples often have a well-fixed outer rim, with a poorly fixed inner core of tissue. In this study, we show that the application of elevated pressure up to 15,000 psi improves the rate of formaldehyde fixation by approximately 5 to 7-fold while preserving the tissue morphology of porcine liver. The tissue also exhibited much more uniform formaldehyde penetration after 30-60 min incubation under elevated pressure than samples fixed for the same length of time at atmospheric pressure.

Discussion

Formalin-fixed, paraffin-embedded tissue is frequently used to preserve specimens for pathological examination due to the high quality of the resulting tissue blocks, its low cost and ease of archival storage [2]. However, the slow rate of passive formaldehyde diffusion necessitates longer tissue processing times of up to 18 hr for routine biopsy samples. In this study, we demonstrate that the addition of elevated pressure to a standard formaldehyde-fixation protocol increases the rate of diffusion of 10% neutral buffered formalin. The apparent formaldehyde diffusion coefficient, *K*, increased by 5-fold for tissue incubated with pressure pulsing ($\Delta 5,000$ psi), and 7-fold at 42°C, and 15,000 psi. Tissue morphology and cellular structure were well preserved for samples fixed at elevated pressure for as little as 15 min, and were comparable to tissue fixed at atmospheric pressure for 4 or more hours. In contrast, tissue incubated in formaldehyde at ambient pressure for short periods of time appeared to be in poorer condition, most likely due to the poor penetration of formaldehyde. These studies show that the addition of elevated pressure to conventional formaldehyde fixation improves the diffusion of formaldehyde throughout the tissue. It is possible that elevated pressure can be applied to all steps of histology to reduce total tissue processing time and expedite pathology results.

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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. Such forward looking statements include statements regarding the expectation that the Company's development efforts funded by the Phase I grant from the NIH will be successful and may lead to the submission of a Phase II grant, and the expectation that the Company will receive the Phase II grant and the expected amount of such Phase II funding; the expected benefits of the CRADA with AFIP, ARP, and the VA, including their collaboration with PBI in this grant, and the possibility of developing pressure-based methods to improve the quality and speed of FFPE tissue preparations; the potential benefits, improvements, and results of using pressure to enhance FFPE processing; the use of FFPE for the preservation of tissues for pathology evaluation; the problems associated with current methods for tissue fixation; the number of FFPE samples processed yearly; and that improved quality in the preparation of FFPE tissues could lead to better results, faster disease diagnosis, and the development of new diagnostics, therapeutics, and vaccines. 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: possible difficulties or delays in the implementation of the Company's strategies that may adversely affect the Company's continued commercialization of its PCT-based product line; changes in customer's needs and technological innovations; the Company's sales force may not be successful in selling the Company's PCT product line because scientists may not perceive the advantages of PCT over other sample preparation methods; that the Company may not be successful in raising the additional capital necessary to fund the Company's operations beyond early October; and if actual operating costs are higher than anticipated, or revenues from product sales are less than anticipated, the Company may need additional capital sooner than expected. Given the uncertainty in the capital markets and the current status of the Company's product development and commercialization activities, there can be no assurance that the Company will secure the additional capital necessary to fund its operations beyond early October on acceptable terms, if at all. Additional risks and uncertainties that could cause actual results to differ materially from those indicated by these forward-looking statements are discussed under the heading "Risk Factors" in the Company's Annual Report on Form 10-K for the year ended December 31, 2010, 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.

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

<http://www.pressurebiosciences.com>

<http://bit.ly/pQRPCp>