PBI Pressure BioSciences Inc.

PCT NEWS

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Pressure BioSciences' PCT Platform Results in a Promising New Method for Improved Extraction of DNA from Rape Kits and other Forensic Samples

Novel PCT-enhanced Method for Selective Extraction of DNA from Male or Female Cells Collected on Cotton Swabs Published in Major Scientific Journal

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South Easton, MA, July 7, 2015 – Pressure BioSciences, Inc. (OTCQB: PBIO) ("PBI" and the "Company"), a leader in the development and sale of broadly enabling, pressure cycling technology ("PCT")-based sample preparation solutions to the worldwide life sciences industry, today announced the development of a two-step, PCT-enhanced method for the selective differential extraction of male or female DNA from mixtures of sperm and vaginal epithelial cells recovered from cotton swabs. The results of the study were recently published in *Analytical and Bioanalytical Chemistry*, a leading peer-reviewed scientific journal. The authors of the paper were Dr. Deepthi V. Nori and Dr. Bruce R. McCord of the Department of Chemistry, Florida International University ("FIU").

For years, biological samples for forensic analysis (e.g., body fluids) have been collected using cotton swabs. Unfortunately, sample recovery (e.g., cells, DNA) from these swabs can be cumbersome, time-consuming, difficult, and expensive. Often the biological sample is trapped in the swab's cotton fibers, resulting in a loss of precious evidence. Over the years, there have been many attempts to develop new methods to improve the speed and quality and to reduce the cost of recovering biological samples from cotton swabs, including the recovery of DNA from swabs taken after a sexual assault.

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UPCOMING EVENTS

Pressure BioSciences, Inc. (PBI) to showcase its new cutting-edge Barozyme HT48 high throughput instrument for enhanced enzymatic digestion of proteins and PCT-HD for both protein extraction and digestion from biopsy-sized tissue samples at the HUPO 14th Annual World Congress (HUPO 2015), September 27 – 30, 2015, Vancouver Convention Center, Vancouver, British Columbia, Canada

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The application of alkaline lysis and pressure cycling technology in the differential extraction of DNA from sperm and epithelial cells recovered from cotton swabs

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Abstract
This study reports the development of a two-step protocol using pressure cycling technology (PCT) and alkaline lysis for differential extraction of DNA from mixtures of sperm and vaginal epithelial cells recovered from cotton swabs. In controlled experiments in which equal quantities of sperm and female epithelial cells were added to cotton swabs, 5 minutes of pressure pulsing in the presence of 0.4 M NaOH resulted in 104 ± 6% recovery of female epithelial DNA present on the swab. Following the pressure treatment, exposing the swabs to a second 5 minute alkaline treatment at 95ºC without pressure resulted in the selective recovery of 69± 6 % of the sperm DNA. The recovery of the vaginal epithelia and sperm DNA was optimized by examining the effect of sodium hydroxide concentration, incubation temperature and time. Following the alkaline lysis steps, the samples were neutralized with 2 M Tris (pH 7.5) and purified with phenol-chloroform-isoamyl alcohol to permit downstream analysis. The total processing time to remove both fractions from the swab was less than 20 minutes. Short tandem repeat (STR) analysis of these fractions obtained from PCT treatment and alkaline lysis generated clean profiles of female epithelial DNA and male sperm DNA for 1:1 mixtures of female and male cells, and predominant male profiles for mixtures up to 5:1 female to male cells. By reducing the time and increasing the recovery of DNA from cotton swabs, this new method presents a novel and potentially useful procedure for forensic differential extractions.

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Dr. McCord, Associate Director of FIU's International Forensic Research Institute ("IFRI") and principal investigator of the study, said: "Our study presents a novel technique to extract biological fluids from cotton swabs with high recovery, using PCT combined with alkaline lysis. This new method has shown decreased processing times and higher yields while using inexpensive reagents on a semi-automated platform. Although there is still work to be done, results to date indicate this PCT-based technique is a promising new method for extraction of DNA from forensic evidentiary materials, including rape kits."

News reports point to a current national backlog of more than 400,000 untested rape kits, sitting in storage and awaiting processing and analysis. Unfortunately, each untested kit may be allowing a rapist to remain free. New sexual assault cases, estimated at 180,000 annually, tragically add to this backlog.

Mr. Richard T. Schumacher, President and CEO of PBI, said: "We believe this novel, PCT-dependent method represents a potential breakthrough in the recovery of high quality DNA from cotton swabs. Next steps will be to optimize and streamline the method. To that end, we have begun discussions to formalize the relationship between PBI and Dr. McCord/FIU into a mutually-beneficial collaboration agreement. We believe that by combining capabilities, knowledge, and our shared interest in an effective outcome, we can more efficiently move this method down the path to potential commercialization."

Mr. Schumacher continued: "Future commercialization of this novel, PCT-based method could potentially provide significant new business channels for PBI in important forensic applications, such as touch DNA and rape kit testing. We believe the forensics market consists of hundreds of labs in the US, performing thousands of tests on a routine basis. Thus, in addition to instrument sales, we also see the potential for single-use consumables and other reagents that are proprietary to our PCT platform."

About Florida International University

Florida International University ("FIU") is recognized as a Carnegie engaged university. It is a public research university with colleges and schools that offer more than 180 bachelor's, master's and doctoral programs in fields such as engineering, international relations, architecture, law and medicine.

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The application of alkaline lysis and pressure cycling technology in the differential extraction of DNA from sperm and epithelial cells recovered from cotton swabs: Continued from Page 1, Column 2

MATERIALS AND METHODS

Pressure Cycling Technology

A Barocycler® NEP 2320 (Pressure BioSciences Inc., South Easton, MA) was used to generate cycles of high pressure and ambient pressure to apply mechanical stress on the cells and cause lysis. There are four main parameters that can be varied to achieve an optimum environment for cellular disruption. The applied pressure, exposure time, and number of cycles can all be adjusted using a programmable interface. The Barocycler™ NEP2320 (Pressure BioSciences Inc., Boston, MA) is a commercially available instrument that is equipped with a hydrostatic pressure chamber that can withstand high pressures up to 45,000 psi and samples can be exposed to target high pressure or ambient pressure from 1 to 99 seconds. Cycling between high pressure and ambient pressure can be repeated up to 99 times. Both solid tissue and liquid samples can be processed using this instrument. Furthermore, connecting an external circulating water bath can control the temperature of the pressure chamber. The instrument is connected to a compressor that creates high pressure. PULSE™ tubes (Pressure BioSciences Inc., South Easton, MA) are specially designed tubes that are able to withstand high pressures. To perform pressurized extraction, swabs containing mixtures of sperm cells and vaginal epithelial cells were transferred to PULSE™ Tubes and exposed to 10,000 psi, 20,000 psi or 45,000 psi in the presence of a lysis buffer to determine the optimum pressure to achieve selective lysis of female cells. The efficiency of the pressure treatment in the presence of increasing number of cycles was studied by varying the cycle number between 10, 20 and 60 cycles. During each pressure cycle, the holding time of sample at ambient pressure (T1) and target pressure (T2) was 15 seconds each.

Alkaline lysis

Hudlow et al. developed an alkaline-based differential extraction method that, when combined with DNase digestion, generated a purified sperm fraction as determined by STR genotyping [15]. Upon applying this method and quantifying the extracted DNA, it was observed that there was a significant loss of sperm DNA. This led us to initiate a study to determine the effect of alkaline lysis on sperm cells and vaginal epithelial cells without DNase digestion at different temperatures and concentrations of sodium hydroxide.

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About Florida International University (continued)

As one of South Florida's anchor institutions, FIU contributes $8.9 billion each year to the local economy. FIU is Worlds Ahead in finding solutions to the most challenging problems of our time. FIU emphasizes research as a major component of its mission. FIU has awarded over 200,000 degrees and enrolls more than 54,000 students in two campuses and three centers including FIU Downtown on Brickell, FIU@I-75, and the Miami Beach Urban Studios. FIU also supports artistic and cultural engagement through its three museums: the Patricia & Phillip Frost Art Museum, the Wolfsonian-FIU, and the Jewish Museum of Florida-FIU. FIU is a member of Conference USA and has over 400 student-athletes participating in 18 sports. For more information about FIU, visit http://www.fiu.edu.

About the International Forensic Research Institute

The International Forensic Research Institute ("IFRI") serves local and national law enforcement efforts in the application of scientific principles to the administration of justice. The mission of this self-supporting institute is to conduct original research in forensic science, transfer technologies from other scientific disciplines for use in forensic applications, provide scientific expertise to the law enforcement and legal community and to provide needed continuing education and advanced training to practicing scientists. IFRI provides undergraduate, master, and doctoral level programs in forensic science.

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 forensic 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, 2014, 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 website link:

http://www.pressurebiosciences.com

Please visit us on Facebook, LinkedIn, and Twitter.

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Alkaline lysis (continued)

Sodium hydroxide crystals (Fisher Scientific, NJ) were dissolved in molecular biology grade water (Fisher Scientific, NJ) to achieve concentrations of 0.2 N, 0.4 N, 0.6 N, 0.8 N and 1 N NaOH concentration. In order to maximize DNA recovery from cotton swabs, three different incubation temperatures (75°, 85°, and 95° C) and two different incubation times (2 minutes and 5 minutes) were studied for each concentration of sodium hydroxide solution. A cotton swab containing an equal quantity of sperm cells and epithelial cells was suspended in 400 µL of a specific concentration of sodium hydroxide solution and exposed to either 75°, 85°, or 95° C for 2 or 5 minutes.

Concluding remarks

The current study presents a novel technique to extract biological fluids from cotton swabs with high recovery using pressure cycling combined with alkaline lysis, and has further demonstrated its potential for differential extraction. With a total of time of less than 20 minutes to remove both sperm and epithelial cells from spiked swabs, this new process is quick and efficient. Current methods for removal of cellular debris from swabs require an incubation time of 2-4 hours, which does not include the differential DNA extraction. By using sodium hydroxide solutions for cell lysis and phenol-chloroform-isoamyl alcohol to purify the extracted sample, we have demonstrated that by using simple solvents available in every lab, sperm and epithelial fractions could be successfully separated in a rapid and efficient manner. Despite the promising results, this study requires further exploration to minimize female DNA carryover observed in samples overwhelmed with high ratios of epithelial to sperm cells. The new method employs a short 5-minute incubation time to lyse female cells that is not sufficient when the sample has a large excess of female epithelial cells. Thus at present laboratories using this procedure would need to monitor the male to female ratio of their samples by real time PCR prior to using this fast protocol. Overall, short extraction times, high yields, inexpensive reagents and semi-automated platform make alkaline lysis-based pressure cycling technology a promising candidate for extracting DNA from forensic evidentiary materials including rape kits.

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