

Pressure BioSciences and Pressure Cycling Technology (PCT) Cited in Journals and at Several Scientific Meetings by Third Party and PBI Scientists

ORTHOPEDIC RESEARCH SOCIETY 2011 ORS ANNUAL MEETING

Mechanical loading-induced TGF- β 1 mediates cartilage degradation

caused by upregulation of HTRA1/DDR2

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INTRODUCTION: The goal of this study is to understand the molecular basis underlying articular cartilage degeneration. Results from our recent investigations suggest that HTRA1 (high temperature requirement A1, a serine protease) degrades the pericellular matrix of chondrocytes, resulting in enhanced exposure of chondrocytes to collagen type II. Interaction of chondrocytes with collagen type II activates DDR2 (discoidin domain receptor 2, a cell membrane receptor tyrosine kinase for native collagen type II). This, in turn, induces expression of MMP-13 (matrix metalloproteinase 13). The end result is osteoarthritis. In this proposed molecular sequence of events underneath articular cartilage degeneration, a critical question concerns which factor(s) cause induction of HTRA1 in chondrocytes. In this study, we tested whether biomechanical factors (hydrostatic pressure and mechanical injury) and/or biochemical factors (TNF α , and TGF- β 1) are implicated in the induction of HTRA1/DDR2.

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CALENDAR OF PBI EVENTS

<u>MASS</u> <u>SPECTROMETRY</u> <u>APPLICATIONS TO</u> <u>THE CLINICAL LAB</u> <u>(MSACL)</u>	<u>AMERICAN ACADEMY</u> <u>OF FORENSIC</u> <u>SCIENCES</u> <u>(AAFS)</u>
FEBRUARY 5-9, 2011	FEBRUARY 21-26, 2011
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PBI's V.P. of Marketing, Dr. Nathan Lawrence Invited to Speak at the 2011 American Laboratory Association (ALA) Meeting

The Challenges of Automating Sample Preparation in the Proteomics Era

Nathan Lawrence and Gary Smejkal

Sample preparation is particularly challenging in proteomics applications. Unlike DNA, which can be amplified, trace recovery of protein risks the exclusion of extremely low abundance proteins of biological significance from the analysis. The efficient release and recovery of the total protein constituencies of tissues and cells thus becomes a critically important initial step in most analytical processes, and is essential to reliable proteomic analyses. However, sample preparation is complicated by the intrinsic diversity of proteins, which can result in bias for or against specific protein subpopulations (e.g. membrane proteins) leading to an inaccurate representation of the proteome. The complexity of proteomes is further compounded by the broad concentration range over which proteins are expressed, as exemplified in human plasma where the mass of albumin is nearly 10 billion times greater than that of cell-signaling proteins like the interleukins.

The disruption of cells and tissues can be accomplished by methods such as sonication, bead beating, homogenization and French press, all of which are disruptive but are capable of generating heat and denaturing the proteins. This is undesirable for downstream applications where protein conformation and biological activity need to be preserved (e.g. the disruption of epitopes required for antibody recognition). The use of intermittent cooling to offset heat during sample preparation may produce thermal fluctuations that, if not carefully controlled, may compromise experimental reproducibility and have deleterious effect. A pressure cycling technology (PCT) platform has been described that uses pressures of 5,000 to 45,000 psi without imposing sheer forces. The rapid oscillation between two pressures has been shown to be more disruptive to cells than sustained high pressure. Moreover, pressure cycling exploits opposing adiabatic processes, since rapid decompression is an exothermic process which offsets temperature increases resulting from compression.

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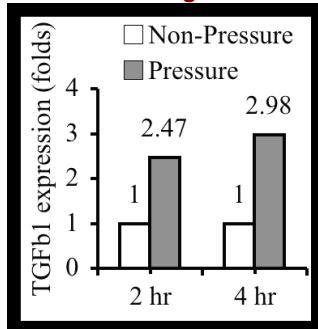


Figure 3. TGF β 1mRNA in chondrocytes was increased by ~3-fold after 2 and 4-hrs of hydrostatic pressure

DISCUSSION: TGF- β 1 is considered an anabolic factor that may act during the early stage of OA by stimulating ECM production. However, data from our present study indicated that

- 1) hydrostatic pressure increased expression of TGF- β 1 in chondrocyte cultures.
- 2) mechanic loading induced and activated TGF- β 1 signaling in articular cartilage of knee joints of a mouse model of OA.
- 3) TGF- β 1 induced expression of HTRA1, which could accelerate cartilage pericellular matrix degeneration.
- 4) mechanic injury of bovine explants caused an increase in expression of DDR2.

We previously reported that mechanical injury of bovine explants caused a significant increase in TGF- β 1 gene expression. These data together suggest a direct link between mechanical stimuli and gene and protein expressions of HTRA1 and DDR2.

CONCLUSION: Mechanical loading-induced TGF- β 1 can mediate cartilage degradation by upregulation of HTRA1/DDR2.

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THE ASSOCIATION OF MASS
SPECTROMETRY APPLICATIONS TO THE
CLINICAL LAB (MSACL) 2011 MEETING**

Ms. Deepthi Nori
(Florida International University)
Invited to Present at the
American Academy of Forensics
AAFS 2011 Annual Meeting

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

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

After attending this presentation, attendees will understand a new method for the differential extraction of DNA from sperm and epithelial cells in sexual assault casework. This presentation will provide the forensic community with a better understanding of how pressure cycling technology can be used to speed up and simplify the extraction process.

One of the stumbling blocks in obtaining a successful male genetic profile in sexual assault cases involves the separation of the evidence left behind by the perpetrator from that of the victim's. Conventional differential extraction method used for the separation of DNA from sperm and epithelial cells is time consuming and requires expertise. It is imperative to develop a method that addresses the issues of time, efficiency and ease of use.

Pressure cycling technology sample preparation system (PCT SPS) is a novel method that involves the use of pressure to disrupt tissues, cells and cellular structures enabling the recovery of their components. In this research we have utilized a commercially available instrument from Pressure Biosciences with a hydrostatic pressure chamber that generates alternating cycles of ambient and high pressure up to 35000 psi resulting in the lysis of cells. Sample cells are placed in liquid suspension in microtubes and subjected to a range of on and off pressure pulses in an attempt to isolate and recover DNA. The microtubes are made from a fluoropolymer that renders them chemically resistant to improve sample recovery and limit adsorption.

The current study involves the application of pressure cycling technology in the extraction of nucleic acids from sperm cells and vaginal epithelial cells. The cells were suspended in 1X PBS buffer (pH 7.4) and subjected to 5,000 psi- 35,000 psi pressure in increments of 5,000 psi accompanied by varying number of cycles to determine the conditions at which one type of cell could be lysed differentially over the other. Samples were placed in microtubes and introduced into the pressure chamber.

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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 Alu-based real-time PCR method using SYBR green.

Our initial studies indicate the potential of PCT application in analyzing samples from sexual assault cases in particular indicating improved extraction of sperm DNA at high pressures when compared to epithelial cells. Overall these results provide new opportunities to explore the ability to generate male DNA profile by selectively lysing sperm cells from mixtures.

RESEARCH ARTICLE: *Proteomics* 2011, 11, 309–318 Rapid and efficient protein digestion using trypsin coated magnetic nanoparticles under pressure cycles

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Trypsin-coated magnetic nanoparticles (EC-TR/NPs), prepared via a simple multilayer random crosslinking of the trypsin molecules onto magnetic nanoparticles, were highly stable and could be easily captured using a magnet after the digestion was complete. EC-TR/NPs showed a negligible loss of trypsin activity after multiple uses and continuous shaking, whereas the conventional immobilization of covalently attached trypsin on NPs resulted in a rapid inactivation under the same conditions due to the denaturation and autolysis of trypsin. A single model protein, a five-protein mixture, and a whole mouse brain proteome were digested at atmospheric pressure and 371C for 12 h or in combination with pressure cycling technology at room temperature for 1 min. In all cases, EC-TR/NPs performed equally to or better than free trypsin in terms of both the identified peptide/protein number and the digestion reproducibility. In addition, the concomitant use of EC-TR/NPs and pressure cycling technology resulted in very rapid (<1 min) and efficient digestions with more reproducible digestion results.

Concluding Remarks

The combination of PCT and EC-TR/NPs appears effective for a fast and efficient protein digestion. The trypsin coating aggregated and crosslinked onto magnetic NPs represents a promising strategy for high-throughput platforms. The high stability obtained allows for an easy storage and continuous use at room temperature for an extended period, and the magnetic capture of EC-TR/NPs is extremely useful for their easy recovery when using robotic stations in a 96-well plate format. Furthermore, by using EC-TR/NPs under pressure cycles, the protein digestion is significantly accelerated, eliminating the need for long incubation times, and the excellent mixing translates into higher digestion reproducibility. These developments provide a basis for fast, robust, and automated sample preparation in support of e.g. high throughput proteomic analyses.

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