Application of Pressure Cycling Technology (PCT) in Differential Extraction

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ABSTRACT

The current study involves the development of a pressure-based lysis method for the differential extraction of DNA from sperm and epithelial cells in sexual assault casework.

Our initial studies indicate the potential of PCT application in generating male DNA profile by selectively lysing sperm cells from mixtures.

INTRODUCTION

Conventional differential extraction methods used for the separation of DNA from sperm cells and epithelial cells are time consuming and require special expertise. These methods rely on selectively digesting epithelial cells, removing them and isolating individual sperm. Unfortunately female cells can overwhelm the process. A better more automated method is needed.

Barocycler® NEP2320, a commercially available instrument from Pressure BioSciences Inc., is equipped with a hydrostatic pressure chamber that can generate alternating cycles of ambient and high pressures up to 45000 psi capable of lysing cells and cellular structures.

Figure 1. Barocycler® NEP2320

Our goal is to utilize the Pressure Cycling Technology Sample Preparation System (PCT-SPS) to selectively lyse sperm cells while keeping epithelial cells intact when analyzing sexual assault evidence.

MATERIALS AND METHODS

SAMPLES
Sperm cells and Epithelial cells suspended in 1X PBS buffer (pH 7.4)

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CELL LYSIS
Pressure cycling using Barocycler NEP 2320

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PURIFICATION
Phenol-Chloroform-Isoamyl alcohol extraction

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QUANTITATION
Real-time PCR analysis using Plexor® HY system (Promega Corporation)

RESULTS AND DISCUSSION

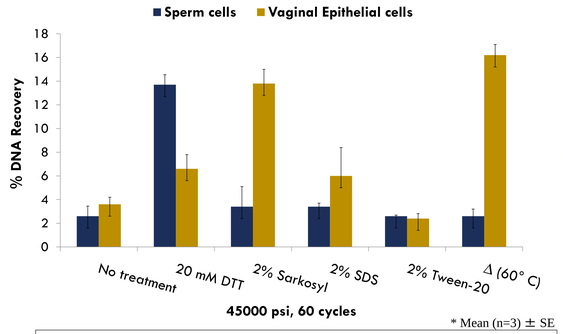

Figure 2. Effect of pressure cycling technology on treated cells

- **Dithiothreitol (DTT)**, a reducing agent, proved to be effective for selectively isolating sperm DNA
- **Sarkosyl**, an anionic detergent, was effective in the selective lysis of epithelial cells, especially with increased extraction temperature.
- Thus the method can be chemically tuned to provide specific recovery of different cell types.

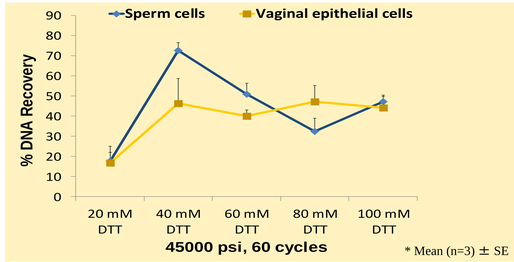

Figure 3. Effect of pressure cycling technology on cells treated with 20, 30, 40, 50 and 100 Mm DTT

- The concentration of **DTT** affects cell selectivity and recovery with best results around 40 mM, however selectivity still needed improvement. We examined other reducing agents.

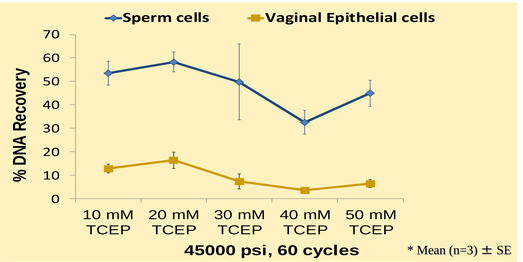

Figure 4. Effect of pressure cycling technology on cells treated with Tris (2-carboxyethyl) phosphine (TCEP), a reducing agent

- Switching to **TCEP** increased the recovery of DNA from sperm cells and produced an increase in selectivity between sperm cell and epithelial cell lysis

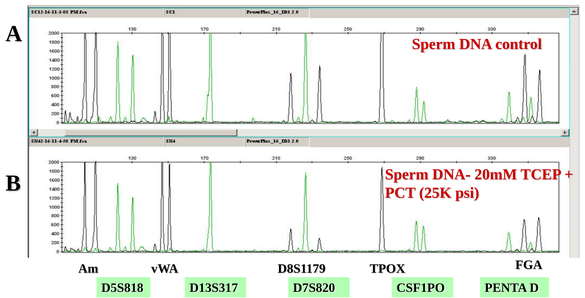

Figure 5. PowerPlex® 16 HS products of sperm cells using PCIA extraction (A) or Pressure cycling with TCEP(B)

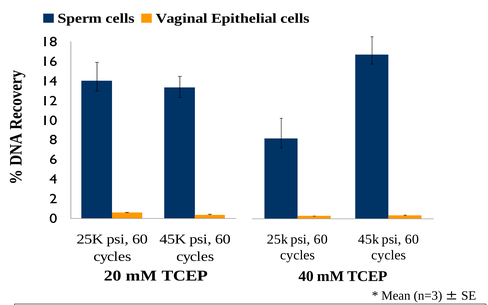

Figure 6. Effect of pressure and [TCEP] on the selectivity of cell lysis. Best results at high P and elevated TCEP

- Acceptable recovery and potential for different lysis is observed with Tris (2-carboxyethyl) phosphine (TCEP) treatment even at lower pressure treatment
- Sperm DNA recovered from pressure lysis was successfully amplified using PowerPlex®16 HS system

CONCLUSIONS

- Pressure Cycling Technology (PCT) has been shown to have potential application in differential extractions.
- The high selectivity and improved recovery with the reducing agent, Tris (2-carboxyethyl) phosphine (TCEP) indicates the potential for highly selective detection of sperm cells.
- Further studies will need to be done to maximize the relative amount of DNA recovered from mixtures and dried stains.

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FOR FURTHER INFORMATION

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