Application of Pressure Cycling Technology (PCT) in Differential Extraction

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Introduction

Conventional differential extraction methods used for the separation of DNA from sperm cells and epithelial cells are time consuming and require special expertise. It is imperative to develop a method that addresses the issues of time, efficiency and ease of use.

The current study involves the application of pressure cycling technology sample preparation system (PCT SPS) in the extraction of nucleic acids from sperm cells and vaginal epithelial cells .

Objective

Our goal is to determine the possibility of selectively lysing sperm cells while keeping the epithelial cells intact from mixtures in sexual assault evidence.

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 35000 psi (with an upgrade option to 45000 psi) resulting in the lysis of cells and cellular structures enabling the recovery of their components.

A

Figure 1.(A) Barocycler® NEP2320 (B) PULSE™ Tubes (Pressure Used to Lyse Samples for Extraction) (C) PCT MicroTubes

Materials and Methods

Sample preparation

Vaginal epithelial cells were extracted from a swab in 1X PBS buffer (pH 7.4). Cell enumeration was done using a hemocytometer (2.5-3.0 x 10^5 cells/ml)

Semen samples were diluted by 20 times with 1x PBS buffer (pH 7.4) to obtain a final concentration of 2.5-3.0 x 10^5 cells/ml.

Pressure cycling: Samples placed in PCT MicroTubes were introduced into the pressure chamber and subjected to varying cycles of pressure in an attempt to differentially disrupt cells.

Purification: Standard phenol-chloroform-isoamyl alcohol extraction was performed following pressure lysis.

Quantification: Plexor® HY system (Promega Corporation) was used to perform real-time PCR analysis to estimate the quantity of DNA recovered from the samples.

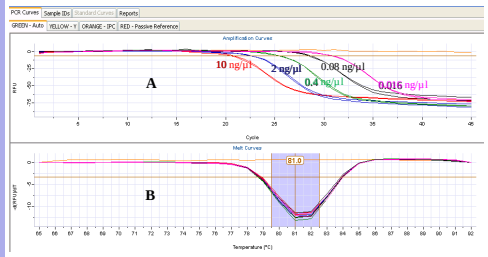

Figure 2. Plexor® HY Male Genomic DNA Standard Amplification Curves, from 10 ng/µl to 16 pg/µl (A) Amplification curves show the relative fluorescence units (RFU) at each cycle of the reaction. (B) A reproducible melt curve (dissociation temperature, Tm) for each sample confirms the amplified product is from the intended target.

Results and Discussion

•Total DNA in each sample was determined using no pressure treatment and standard Proteinase K extraction.
•Our first step was to gauge the response of sperm cells and vaginal epithelial cells when exposed to high pressures without the aid of extraction reagents.
•Microscopic visualization of cells stained with 0.4% Trypan blue solution indicated loss of membrane integrity in some cells subjected to pressure cycling technology.

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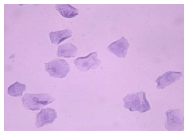

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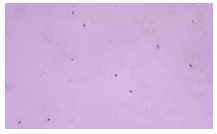

Figure 3. PCT-treated cells stained with 0.4% Trypan blue solution and observed under 40X magnification of a compound microscope. Cells with a compromised membrane integrity are stained dark blue. (A) Vaginal epithelial cells (B) Sperm cells

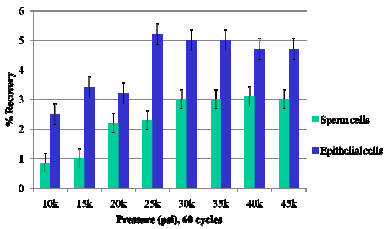

Figure 4. Effect of pressure treatment on sperm cells and vaginal epithelial cells.

The extraction efficiency from pressure lysis is plotted as percent recovery of DNA from the samples. It can be observed from the graph that:

• DNA was recovered from both the samples
•Extraction efficiency was low

Our next step was to maximize DNA recovery from the samples. To this effect, the samples were pressure cycled in the presence of different detergents with a final concentration in the sample as summarized in the table below

Detergent	Concentration	Pressure (psi)	No. of cycles
DL-Dithiothreitol (DTT)	20 mM	45000	60
Sodium lauroyl sarcosinate	2%	45000	60
Sodium Dodecyl sulfate	2%	45000	60
Tween 20	2%	45000	60

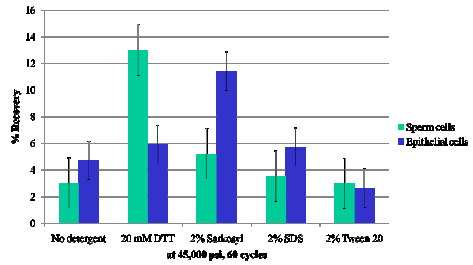

Figure 5. Effect of pressure treatment on treated cells. DTT treatment proved to be effective for DNA extraction from sperm cells whereas Sarkosyl treatment was most effective in the lysis of epithelial cells.

It can be observed from the graph that DTT is more effective in the lysis of sperm cells whereas an increase in recovered DNA was observed with Sarkosyl treatment of epithelial cells.

Conclusions and Future work

•Pressure Cycling Technology (PCT) has been shown to have potential application in differential extractions.

•An increase in extraction efficiency is observed with sperm cells treated with Dithiothreitol, while improved extraction of epithelial cells is possible using detergent.

•Further studies need to be done to maximize the relative amount of DNA recovered from the sperm cells which will enable us to perform selective lysis of sperm cells in the presence of epithelial cells.

Acknowledgements

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References

1)Aravindan, G.R., Krishnamurthy, H., and Moudgal, N.R. (1997) *J Androl.* 18: 688-697.
2)Evenson, D.P., Darzynkiewicz, Z., and Melamed, M.R. (1980) *Chromosoma (Berl.)* 78: 225-238
3)Motoishi, M., Goto, K., Tomita, K., Ookutsu, S., and Nakanishi, Y. (1996) *J Reprod Dev.* 42 (1): 7-13.
4)Norris, J.V., Manning, K., Linke, S.J., Ferrance, J.P., and Landers, J.P. (2007) *J Forensic Sci.* 52 (4): 800-805
5)Somfai, T., Bodó, Sz., Nagy, Sz.,Gócza, E., Iváncsics, J., and Kovács, A. (2002) *Biotech Histochem.* 77 (3): 117-120.
6)Tao, F.; Li, C.; Smejkal, G.; Lazarev, A.; Lawrence, N.; and Schumacher, R.T. (2007) The 4th International Conference on High Pressure Bioscience and Biotechnology. 1: 166–173.
7)Plexor® HY system, *User manual*, Promega Corporation.

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