B105 Enzymatic-Mediated Digestion of Cellulose for Enhanced Cell Elution for DNA Analysis

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The focus of this project is the development of an improved method for the elution of cells from a cotton swab taken from a sexual assault victim. The procedure incorporates enzymes for the digestion of the cellulose matrix, resulting in the removal of intact cells, in an effort to circumvent conventional differential extraction.

Genetic analysis of perpetrator and victim mixed profile DNA samples obtained from vaginal swabs is a well-established technique in the investigation of sexual assault and rape cases. Unfortunately, the procedures involved in a typical forensic DNA analysis require a great deal, of laboratory time dedicated to a single case, particularly in the sample preparation steps. Because of the time and funding constraints involved in the investigation of such cases, a significant backlog exists in many largevolume DNA analysis laboratories.

The current protocol used by law enforcement agencies for recovery of cellular materials from a cotton matrix involves a great deal of sample handling, which directly increases the chances of sample contamination and human error. Furthermore, it is a time-consuming process often requiring overnight incubation of a swab sample for optimal DNA recovery. The extraction solution used in the recovery of DNA from swabs includes proteinase K in the presence of SDS, a combination that selectively lyses the fragile epithelial cells while eluting sperm cells intact. The solution is then centrifuged to pellet the sperm cells, removing them from the solution containing the victim DNA, allowing independent genetic analysis of male and female DNA.

The time required for forensic DNA analysis can be greatly reduced by performing the electrophoretic separation on microfabricated glass devices. The speed and efficiency of microchannel separations are due largely to the increased surface to volume ratio of the etched-channels over conventional slab gels. In addition, these devices allow for the integration of additional processing steps, including sample preparation methods. Because centrifugation on a microchip is not trivial, a microchip method for isolating separate male and female DNA fractions has been proposed. This method relies on recovery of intact cells from sample swabs, thus a cell-desorption process that greatly reduces extraction time while leaving cells intact would be advantageous for developing genetic analysis on a micro-total analysis system (μ-TAS).

Microscopic examination of a cotton swab on which a semen sample had been applied and allowed to dry suggested that sperm retention on the swab was due to adhesion of sperm cells on the surface of polysaccharide cellulose strands. Preliminary studies have shown that cellulase-based enzymes, which digest cellulose, reduce the time required for sperm and epithelial cells to be released from the swab into solution. In an effort to optimize cellular elution conditions, several different enzymes were evaluated both alone and in combination. Sperm and epithelial cells eluted from a cotton swab were counted using a hemacytometer. Results indicate that elution using enzymes improved the recovery of sperm cells without lysing epithelial cells, and enzymatic sperm cell desorption is greater than that seen with current elution methods. Optimum cellular elution conditions using the enzyme cellulase will be presented. In addition, information regarding the development of a receptacle that interfaces a cotton swab sample with a μ-TAS on a microfabricated glass device will be discussed.

Cellulose, Elution, Enzyme

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