A100 Towards a Microfluidic Device for Integrated Purification and Amplification of RNA

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After attending this presentation, attendees will gain an understanding of the development and applications of a microdevice that can process a biological sample by first isolating and purifying RNA followed by RT - PCR amplification.

This presentation will impact the forensic community by introducing a microdevice for integrated purification of RNA and RT-PCR analysis for forensic body fluid identification. This device will allow for faster evidential analysis and increased throughput for forensic casework sample processing. The methodology greatly reduces the number of sample transfer steps and is performed in the closed environment of a microfluidic device, reducing the potential for contaminants and RNases to enter the system and degrade a sample. This work is another step towards the realization of a micro-total analysis system for body fluid identification using mRNA expression analysis. The purpose of this research is to demonstrate the development of a single microfluidic device for the integrated solid phase purification and subsequent RT-PCR amplification of RNA for forensic body fluid identification.

mRNA expression analysis is based on inherently variable mRNA expression from different cell types, producing gene-specific patterns[1] which can verify the presence of individual body fluids present in a forensic sample.[2] The importance of body fluid identification in an investigation is realized when it is necessary to determine the number of contributors in a sample, as well as when a body fluid, in the form of a stain, originates from a single person. The origin of each body fluid can be determined, providing important information to investigators clarifying the criminal act which took place.

Prior to analysis, RNA from a biological sample must be isolated. Reverse transcription-PCR amplification and separation of target amplicon can then be performed to identify the sample. Conventional methods for RNA extraction do not guard against the inherent sensitivity to degradation and contamination of RNA because they involve many transfer steps exposing the sample to exogenous contaminants and RNases. The use of microfluidic purification systems for DNA has been well-characterized as an alternative to conventional DNA isolation, allowing for reduced sample and reagent consumption, as well as a reduction in analysis time.[3, 4] This technology has also been applied and proven sound for the isolation of RNA from biological samples, providing purified, amplifiable RNA as a result.[5]

In addition to solid phase extraction (SPE), PCR amplification on a microdevice has also been demonstrated, with advantages including smaller reaction volumes (and therefore reduced reagent consumption), possible integration with up and down stream applications, and reduced analysis times.[4, 6] Specifically, the smaller thermal mass associated with microfluidic PCR enables sharp temperature transitions, decreasing not only the time needed for amplification, but also the formation of nonspecific product.[7] Integrated on-chip solid phase extraction (SPE) and PCR for DNA has been shown previously by our group, using infrared-mediated (IR-mediated) heating.[6] Incorporating microchip- based PCR into a system for SPE-RT-PCR for mRNA analysis utilizing IR-mediated heating would greatly benefit the forensic community by providing a closed-system platform for RNA isolation and amplification, reducing sample transfer steps and, therefore, reducing contact of the sample with exogenous contaminants and RNases.

This work proposes to be the first demonstration of a microfluidic system for the silica phase-based purification of RNA integrated with RT- PCR using IR-mediated heating, to reduce thermocycling times, while demonstrating applicability of the system to forensic samples. By integrating the IR heating method for faster RT-PCR with solid phase extraction of RNA, total analysis time would be greatly reduced, making identification of body fluids by mRNA expression more applicable to time-sensitive analyses. Microchip-based SPE (μSPE) will be integrated with microchip-based RT-PCR, utilizing non-contact IR-mediated heating for PCR amplification, bringing the forensic community a step closer towards an integrated micro-total analysis system capable of total mRNA profiling. Experiments detailing studies for optimization of time necessary for reverse transcription as well as cycling times for PCR on a microdevice are included. Also, work towards the extraction of RNA on a microdevice followed by microchip-based RT-PCR amplification will be shown.

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