A47 Towards a Multi-Chamber Plastic Microdevice for Simultaneous Amplification of Multiple DNA Samples

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After attending this presentation, attendees will have gained an understanding of the progress being made towards the development of a multi-sample microfluidic PCR platform for the simultaneous amplification of multiple DNA samples.

This presentation will impact the forensic science community by demonstrating a multi-chamber microdevice capable of amplifying up to seven DNA samples simultaneously, increasing sample throughput as compared to other PCR microdevices and demonstrating the applicability of a microfluidic platform for forensic analyses.

The polymerase chain reaction (PCR) is a key step in the processing of forensic biological samples. Typically, it is the most lengthy step (typically 2.5-3.5 hours) contributing to a time-consuming analytical process on the order of eight to ten hours. However, it has the advantage of high sample throughput, amplifying up to 96 samples at one time with conventional thermal cyclers. Microfluidic platforms for PCR have demonstrated numerous advantages over conventional PCR, including reducing the time required to complete the reaction as well as reducing sample and reagent consumption. Although PCR has been successfully adapted to a microdevice,[1] the majority of devices are limited in that they can only amplify one to two samples at a time. In order for a PCR microchip to be a generally accepted platform, the development of a device capable of amplifying multiple samples at once is clearly important.

Concurrently, the trend in microfluidics is to move towards alternative substrates for microchip fabrication to reduce fabrication and device costs. Typically, microdevices are made from glass, but are time-consuming and laborious to fabricate, often requiring the use of hazardous chemicals. Microdevices from polymeric substrates, such as poly(methyl methacrylate) (PMMA), can be fabricated using simpler techniques, including laser ablation and hot embossing.[2] In addition, PMMA is inexpensive, so devices can be disposed of after a single use and, as a result, this eliminates the risk of contamination between samples.

Previous work has demonstrated the use of infrared-mediated PCR (IR-PCR)[3] which utilizes a halogen lamp and a fan to heat and cool a sample in a PCR microdevice, respectively. IR-PCR provides increased heating and cooling rates compared to conventional block thermal cyclers, therefore reducing the time required for PCR. IR-PCR amplification of short tandem repeat (STR) regions of the human genome in a single chamber PMMA microdevice has been demonstrated using a combination of commercially-available fast polymerases, yielding a full STR profile (16 of 16 loci) in 33 minutes[4] – a ~5-fold reduction from average conventional amplification times.

Multi-chamber PCR microdevices were fabricated in PMMA using a CO_2 laser system, with eight PCR chambers arranged in a circular pattern within a 1cm diameter focal spot of the halogen lamp. The temperature in each chamber was monitored using miniature type-T thermocouples and the inter-chamber temperatures were found to vary up to 4°C, likely due to variation in the emitted radiation from the halogen lamp. Results show that, even though there were significant differences in the peak height of the amplified products (due to the temperature variation), suggesting slight differences in the power delivered to each chamber, STR amplification was successfully performed in the multi-chamber device, yielding full STR profiles (seven of seven loci) in all seven chambers in 42 minutes. With modifications to provide a more homogenous delivery of power from the lamp (i.e., the use of a diffuser), the temperature variations will be minimized, which, in turn, will minimize peak height variations. Furthermore, these results indicate that a polymeric multi-chamber microdevice for simultaneous sample amplification has the potential to increase throughput on a microfluidic platform while reducing overall analysis time as well as cost-per analysis.

References:

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Multi-Chamber PCR, STR Typing, PMMA

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