PMMA Lab-on-a-Chip for RNA Detection

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Abstract:

We describe investigations toward a disposable polymer based chip for the isolation, amplification, and electrochemical detection of eukaryotic mRNA. We introduce a method to fabricate a copper master for rapid prototyping of poly(methyl methacrylate) (PMMA) substrates, as well as a novel means to realize gold interdigitated ultramicroelectrode arrays (IDUA) directly on the PMMA surface without the use of a metal adhesion layer. Finally, we demonstrate the capability of this lab-on-a-chip system to isolate the mRNA of Cryptosporidium parvum and to detect the amplicon from a single C. parvum oocyst.

Summary of Research:

We have been working on efforts to develop a lab-on-a-chip for the detection of specific eukaryotic mRNA. Our first objective was the microfabrication of a sturdy and easily producible hot embossing master for imprinting microchannels into PMMA substrates. This was accomplished by electroplating copper channel molds onto a copper plate patterned with a KMPR negative photoresist (Figure 1). The channel molds had a height of approximately 35 µm high with a variation of ±1 µm. The copper material has a linear coefficient of expansion closer to that of PMMA than silicon which is traditionally used for hot embossed prototypes, and therefore released the substrate much easier.

Microchannels were then made from the copper master which were used for the mRNA isolation of Cryptosporidium parvum. With oligo (dT)25 beads, and an incorporated sawtooth micromixer [1], the device was able to isolate enough hsp70 heat shock mRNA from as few as five C. parvum oocysts to result in a successful nucleic sequenced based amplification off-chip.

The next objective was to incorporate a working IDUA into the microchannel for the detection of the hsp70 amplicon. In order to avoid galvanic reactions, a secondary metal substrate was avoided as the adhesion layer. Instead, the PMMA surface was UV-modified for carboxyl formation, and then conjugated with...
cystamine to provide a thiolated surface. A gold electrode was then patterned on the PMMA using the thiol-gold interaction as an adhesion mechanism. The PMMA containing the IDUA was then UV-bonded [2] to the hot embossed PMMA (Figure 2).

For the amplification detection, superparamagnetic beads tagged with a capture probe were mixed in the channel along with the amplicon from a NASBA reaction. Added to this were liposomes containing potassium ferro/ferricyanide which were tagged with a reporter probe [3]. The bead and liposome formed a sandwich assay with the available amplicon and were then captured by a magnet over the IDUA.

Following a washing step, a detergent was then pumped into the channel to lyse the liposomes and release the potassium ferro/ferricyanide. A 400 mV potential was applied across the electrode and the resulting current was proportional to the bound liposomes.

This device was able to detect the amplicon resulting from a single C. parvum oocyst. Our lab is currently working on developing on-chip NASBA amplification for the final component of the lab-on-a-chip.

References:

