Affinity Separation in Hybrid Polymeric Microfluidic Devices Coupled with Mass Spectrometry

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Abstract

The combination of microfabricated microfluidic devices and mass spectrometry (MS) provides current technologies with significant advantages such as high throughput analysis and fast analysis time [1]. We report the demonstration of affinity separation of proteins in hybrid polymeric microfluidic devices. The microchips were made of cyclic olefin copolymer and obtained through hot embossing and thermal bonding. An SU-8 electrospray tip was patterned and used to interface the microfluidic system with a mass spectrometer. Polymer beads were successfully polymerized, packed in situ and used as a separation matrix when derivatized by Cibacron Blue 3GA. The performance of the microchip was evaluated by MS analysis of the protein sample mixture.

Summary

Photolithography was used to create SU-8 triangular electrospray tips. These tips were sandwiched between two cyclic olefin copolymer chips by using thermal and press bonding. Before bonding, microfluidic channels were embossed in a polymer substrate using a silicon master. After coupling two components, based on a photografting method in situ UV-polymerization was carried out to create an affinity column in the channel. Polymerized beads were then treated with ethylenediamine and derivatized by Cibacron Blue 3GA for 9 hours at 60°C.

For demonstration purpose, the sample mixture of proteins, lysozyme and cytochrome c, were introduced to the device. A microfluidic channel, 150 µm wide and 150 µm deep, was obtained by an embossing method. The SU-8 was spun on the surface of borofloat wafer. After simple lithography, the borofloat wafer was released by HF etching. This SU-8 triangular tip was aligned with the channel and designed to protrude from the end. This triangular structure yielded the formation of a stable electrospray by guiding a Taylor cone along the electric field. For affinity chromatography, the double T-junction was employed to load protein sample mixtures in the main channel. In order to minimize the dead volume from the interconnection between the external pressure source and the microfluidic device, silica capillary tubes were aligned with the embossed channels and delivered proper pressure.

In situ polymerization was achieved by using a photografting method. Monomer and porogenic solvents were introduced to microfluidic channel via silica capillary. A photomask was used and exposed under UV light in order to control the location of the polymer bed. A 5 mm long polymer bed was successfully located at desired location. After the formation of the polymer bed, for on-chip separation using affinity chromatography, this bed was derivatized by Cibacron Blue 3GA [2].

Lysozyme and cytochrome c were selected as sample mixtures. We were able to demonstrate affinity capture of lysozyme in the polymer bed. Mass spectra shows that only cytochrome c has passed through the polymer bed region. An investigation concerning reverse phase liquid chromatography using the same microfluidic device is under way.

References

Figure 1: Total ion current after the affinity capture of lysozyme from the sample mixture. Only Cytochrome C was observed in the peak.

Figure 2: (A, top) Mass Spectrum of bovine Cytochrome C molecular weight of 12231 Da. This protein is introduced to the mass spectrometer by direct infusion using the SU-8 electrospray tip in the circle. (B, bottom)