BEAD BASED TEMPERATURE CONTROLLABLE MICROCHIP FOR CANCER DIAGNOSIS

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ABSTRACT
This paper reports a novel micro total analysis system for the purification and identification of the affinity-captured proteins. We describe integrated and efficient micro chip for protein purification and digestion for MALDI-TOF analyses. At first, disease protein is purified by passing the micro chamber from a protein mixture or human whole serum and released from the micro affinity beads by thermal heating. Purified protein is then transferred to the hole for trypsin digestion. The final sample is analyzed by MALDI-TOF. All the processes could be finished successfully within one hour, which renders MALDI-TOF analyses of a target protein quite simple.

KEYWORDS: Temperature Control, MALDI-TOF MS, Micro Bead, Protein

INTRODUCTION
Miniaturization of analytical systems is currently considered to be a key technology to overcome the hurdles in qualitative proteome analysis. For this, integration of multiple operations such as sample cleanup, target protein fishing-out, pre-concentration and enzymatic digestion into a chip is important[1, 2]. And mass analyses are a powerful method for identifying proteins. Recently, we have reported micro-fluidic diagnostic methods based on MALDI-TOF analysis of proteins[3]. For MALDI-TOF analyses, the proteins should be separated from a protein mixture and be concentrated when needed. This procedure usually takes a long time even before protease-digested samples are to be obtained from them. We describe here a novel micro total analysis system for the purification and identification of the affinity-captured proteins. Also we demonstrated the mass analysis of the Carcinoembryonic antigen (CEA) and Alpha femtoprotein which were chosen as the target cancer marker.

EXPERIMENTAL
The bead-based temperature controllable micro chip consists of glass cover silicon structure and Pt electrode (Figure 1). The reaction chamber (5.2 μl volume) has a column of posts for bead packing. The Pt electrode on the bottom of the reaction chambers are used for the micro heater and temperature sensor.

Figure 2 has shown the schematic drawing of the preparation of micro affinity bead. Polystyrene based micro bead (40-50 μm) was hydroxyl functionalized by ozone oxidation. Then, the beads were coated with organo-silane group followed by grafting PEG (Polyethyleneglycol) and introducing peptide spacers. The RNA aptamer that we used was targeted to the Carcinoembryonic antigen (CEA) and Alpha
femtoprotein (AFP). After enzymatically modification to primary amine of 3’ tail of RNA aptamers, these aptamers were coupled to the surface of micro beads.

RESULTS AND DISCUSSION

The cancer marker protein detection with affinity micro bead based temperature controllable micro chip and system (Figure 3) was performed as following procedures. And the results of MALDI-TOF MS spectrum were shown Figure 4. First of all, reaction chamber was packed with micro affinity bead. Before using, beads were heated at 85°C for 5 min and cooled for 5 min, then 200 μl of target protein (pure or protein mixture or real patient serum) was circulated into the chamber and then incubated for 30 min at 25°C. After incubation, the chamber was washed with buffer intensively. The capture protein was released from beads and denaturated by applying heat to the chamber at 85°C for 1 min and transferred to next hole. The released protein was digested with trypsin solution for 10 min at 37°C. The digested peptide fragments were eluted and analyzed by MALDI-TOF MS. The results clearly showed several matched peaks. We could acquire over 15 % sequence coverage of cancer marker protein mixture (20 μl human serum and pure protein). And compared the results with normal human serum to confirm that non-specific adsorption of proteins onto the micro beads.

Figure 1. A schematic view and temperature controllable microchip

Figure 2. Preparation scheme of micro affinity bead

Figure 3. Experimental setup of proposed system
CONCLUSIONS

In this work, a bead based temperature controllable micro chip for detection of the CEA and AFP cancer marker prior to the MALDI-TOF MS has been successfully implemented and verified. This system is rapid and efficient in protein purification and identification. Also this system can be integrated for various analytic component such as chromatography, pre-concentration and enzymatic digestion.

ACKNOWLEDGEMENTS

This research has been supported by the Intelligent Microsystem Center, which is one of the 21st century's frontier R&D Projects sponsored by the Korea Ministry of Commerce, Industry & Energy.

REFERENCES

