Microfluidic Biosensor System for HDL Cholesterol

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Abstract

A chromogenic biosensor employing microfluidics on a chip has been developed for the determination of high-density lipoprotein (HDL) cholesterol (HDL-C) in human serum. We have investigated a plain and effective method to immobilize enzymes within the microchip without chemically modifying micro-channel or technically micro-fabricating column reactor and fluid channel network. In assessing risk factors of coronary heart disease, a micro-chip system would minimize requirements of instrument and reagent handling.

Key words: Microfluidic channel, HDL cholesterol, Immuno-separation, Magnetic beads, Chromogenic detection

Introduction

The determination of HDL cholesterol concentration in human blood is needed for the diagnosis and effective treatment of coronary heart diseases (CHD). A significant efforts have been made for developing effective diagnostic tools for the prevention of CHD in recent years. Among various methods for the quantitative measurement of HDL-C, we investigated a generic concept for the measurement of a single class of plasma lipoprotein cholesterol. To this end, an immuno-separation system has been devised such that the separation of plasma lipoproteins on magnetic bio-separator and the generation of a colorimetric signal *in situ*, in proportion to the cholesterol level of the isolated lipoproteins, can be performed simultaneously. The color signal could then be quantified using a portable optical sensor.

In this report, a very simple and efficient method to immobilize various enzymes within microchip devices was developed. NC membrane commonly used as an immobilization matrix for protein was inserted within the structure of chip channels. By introducing such format of enzymes, we developed a novel chromogenic biosensor on a chip utilizing microfluidic channels for the determination of HDL cholesterol in serum.

Materials and Methods

Reagents and standard solution. All chemicals were of analytical reagent grade and deionized water was used for the preparation of solutions. A stock solution of the whole blood and human serum (approximately, 150 mg/dL) was stored at 4℃. Standard solution of cholesterol were prepared daily by diluting the stock solution with delipidated serum. Tetramethyl-benzidine (TMB) solution was prepared by dissolving 0.05 g of TMB in 1 mL of deionized water. Cholesterol oxidase (4 KU/mL), cholesterol esterase (5 KU/mL), and horseradish peroxidase (HRP, 251 U/mg) were obtained from Calbiochem. Nitrocellulose (NC) membrane and magnetic beads were obtained from Whatman and Dynal, respectively.

Instrumentation. A PDMS-PMMA microfluidic system on chip was connected to syringe pump using PTFE tubing (0.2 mm ID). The chromogenic signal produced from the microfluidic system was detected and recorded in a kinetic mode using a computerized Optizen View Analyzer. Data acquisition and management were performed with a software provided by the manufacturer.

Analysis. A MES solution was used as medium to carry sample containing cholesterol by employing a syringe pump. HDL was separated using antibody-coupled magnetic beads under a magnetic field and then decomposed by enzyme-catalyzed esterization and oxidation of cholesterol within the NC membrane pad. H_2O_2 produced was detected using HRP in the presence of a chromogenic substrate. A colored signal in proportion to the concentration of HDL cholesterol

was measured by spectrometry.

Results and Discussion

We employed NC membrane to immobilize enzymes such as cholesterol oxidase and cholesterol esterase, and other analytical reagents such as TMB and HRP. Using this format, enzymes could be stably and efficiently immobilized within the interstitial spaces of the membrane that was eventually placed inside a microchip. The analytical microsystem was composed of four functional compartments connected in a sequence, i.e., immuno-separation based on the antigen-antibody reaction, catalytic conversion of cholesterol to hydrogen peroxide, production of a colorimetric signal, and quantitative measurement using a detector.

For immuno-separation, a monoclonal antibody, specific to apolipoprotein B100 that was present on the surfaces of low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL), with a high binding constant ($5x10^{10}$ L/mol), was raised and chemically conjugated to magnetic beads. The conjugate was first reacted with lipoprotein particles, and this mixture was transferred into immunoseparator of the system and subsequently separated under magnetic force. While LDL and VLDL particles were captured onto immunoseparator, HDL was carried for *in situ* generation of a colorimetric signal in proportion to HDL-C. Such procedure based on antigen-antibody reaction was selective as well as effective, and the detection limit of the HDL-C was far lower than 25 mg/dL.

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