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# On-chip dual detection of cancer biomarkers directly in serum based on self-assembled magnetic bead patterns and quantum dots

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ABSTRACT

A sandwich immunoassay method for rapid detection of dual cancer biomarkers in serum on a magnetic field controllable microfluidic chip (MFCM-Chip) was established. A nickel pattern was used to generate high magnetic field gradients to increase the magnetic force on the superparamagnetic beads (SPMBs), which enabled the rapid generation of controllable SPMB patterns in microfluidic channels. The SPMB patterns could keep stable during the fast continuous washing process even at a flow rate of 50 μL/min. This approach demonstrated excellent specificity because the fast continuous washing could remove non-specifically adsorptive contaminants more efficiently than fixed volume batch washing. This approach was used to simultaneously detect carcinoma embryonic antigen (CEA) and α-fetoprotein (AFP) directly in serums. The whole on-chip detection was finished within 40 min, which was much faster than conventional enzyme-linked immunosorbent assay (ELISA) method. High luminescent streptavidin modified QDs (SA-QDs) were used as fluorescence indicators, and the detection limits were 3.5 ng/mL and 3.9 ng/mL for CEA and AFP, respectively. The linear ranges were from 10.0 ng/mL to 800.0 ng/mL. With the high sensitivity, high selectivity and short assay time, this approach could be used for rapid, high throughput detection of cancer biomarkers in clinical trials.


## 1. Introduction

It is crucial to detect the occurrence of cancer in the early stages, because effective treatment for this can remarkably improve the survival rate of patients. In a routine physical examination, the early diagnosis of cancer is often achieved by screening the biomarkers in patients' serums. So far, many kinds of cancer biomarkers have been reported (Zhou et al., 2006; Baskic et al., 2007; Dungchai et al., 2007). For example, the cancer antigen 125 (CA 125) is measured as a biomarker of the ovarian cancer (Bast et al., 2005; Smith et al., 2011), and the prostate specific antigen (PSA) is related to the prostate cancer (Smith et al., 2011). Meanwhile, simultaneous detection of multiple cancer biomarkers in serum samples is more accurate in the cancer diagnosis than individual biomarker measurements (Lee et al., 2008), because some specific cancers will cause multiple cancer biomarkers overexpressed. The cancer antigen 15-3, CA 125 and human epidermal growth factor receptor 2 (HER 2) are often utilized to diagnose breast cancer (Duffy, 2006; Baskic et al., 2007). However, in clinical practice, cancer biomarkers are usually determined one by one in patient's serum, which is less time efficient. Therefore, it is necessary to develop a simple, rapid and low-cost method for the detection of multiple biomarkers in patients' serums simultaneously, because this could help the doctors accurately diagnose cancer, monitor and assess the treatment effects. Microfluidic chips have the advantages of small dimension, low-sample-cost, fast mass transfer, as well as integration and multiplexing capabilities (Khandurina and Guttman, 2002; Sia and Kricka, 2008), which could meet the above demands.

Magnetic beads as a powerful tool with many properties such as well controlled surfaces, flexible functionalization, easy manipulation by the magnet and large surface-to-volume ratio, were widely applied to improve the selectivity and sensitivity of analysis methods. Due to their efficient enrichment and separation capability, the magnetic beads were successfully utilized in immunoassay (Dungchai et al., 2007; Zhang et al., 2009), DNA hybridization (Li and He, 2009; Cai et al., 2010), virus detection (Zhao et al., 2012), protein analysis (Centi et al., 2007),

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and cell sorting (Smith et al., 2007; Song et al., 2011). Fluorescent and magnetic dualencoded multifunctional bioprobes with different magnetic response were used to recognize and separate multiple lectins (Hu et al., 2011). The conventional bioassay based on magnetic beads was performed in an eppendorf tube with a large reagent-consuming and tedious washing process, which is ineffective (Tennico et al., 2010). The magnetic bead-based assays in microfluidic chips might avoid above drawbacks involved in the conventional methods. Some reports have reviewed the achievements on magnetism and microfluidics (Pamme, 2006; Gijs et al., 2010). Bardea et al. (2009) used magnetic beads to generate magnetolithographic patterns, which could be applied to control the modification of the inner walls of a tube. Chen et al. (2011) and Liu et al. (2007b) used the permanent magnets and electromagnets to generate magnetic bead plugs to perform immunoassays in microfluidic chips. However, the magnetic flux density was not concentrated and the magnetic bead plugs would be destroyed at high flow velocity, which was not suitable for rapid assay.

The multiplexed detection might be simply performed in microfluidic chips with parallel branch channels and self-assembled SPMB patterns. Three plugs of magnetic particles were immobilized with external magnets (Bronzeau and Pamme, 2008) and used for simultaneous bioassays in a microfluidic chip. Sivagnanam et al. (2009) utilized electrostatic assembly of streptavidin-coated bead patterns to detect mouse IgG and rabbit IgG in a microfluidic chip with two branch channels. As in our previous report (Yu et al., 2011), we have demonstrated a method by using a nickel pattern encapsulated in a thin PDMS to induce high magnetic field gradients and generate SPMB patterns, which could be used for multiplexed detection.

Quantum dots (QDs) were inorganic fluorescent materials owning many unique properties such as high quantum yield, broad absorption, narrow emission spectra and excellent photostability (Chan and Nie, 1998; Peng et al., 2000) were widely applied in biomedical imaging and detection (Xing et al., 2007; Kang et al., 2009; Xie et al., 2010; Algar et al., 2011).

AFP was a 70 kD glycoprotein expressed abundantly in fetal liver, but not in normal adult liver (often less than 10 ng/mL) (Meany et al., 2009; Yang et al., 2010). However, it overexpressed in the serum of patients who obtained cirrhosis or hepatocellular carcinoma (HCC). AFP was widely utilized for early screening and monitoring high-risk patients for HCC (Hippo et al., 2004). CEA was a cell surface glycoprotein expressed normally in adult and fetal intestine (normally less than 5 ng/mL) (Jokerst et al., 2009; Yang et al., 2010), but it commonly overexpressed in the serum of patients with some tumor diseases including colorectal cancer, gastric cancer, and lung cancer (Fu et al., 2007). Therefore, CEA was applied in early screening and clinical diagnosis of these cancers.

In this paper, we developed a SPMB pattern-based immunoassay method for dual detection of cancer biomarkers (AFP and CEA) in microfluidic chips. The nonspecific adsorption could be efficiently eliminated by fast continuous washing in microfluidic channels, and the high sensitivity and selectivity detection of cancer biomarkers in serums could be realized. Moreover, the whole sandwich immunoassay process could be completed in 40 min. And through a simple ultrasonic washing process, the robust MFCM-Chip could be renewed and reused repeatedly for many times. Finally, this approach could be readily applied for simultaneous detection of other types of targets, such as viruses, bacteria and so on.

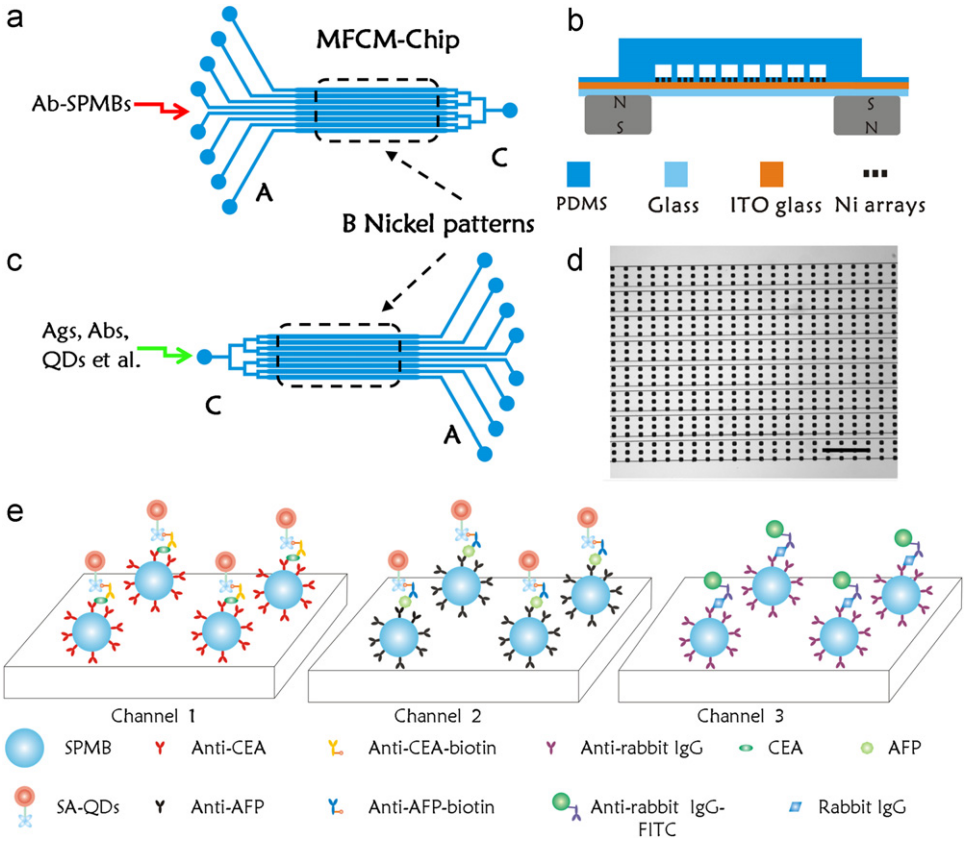


**Fig. 1.** Schematic diagrams of an integrated MFCM-Chip and sample loading process. (a) Scheme of Ab-SPMBs loading process. (b) The cross-sectional view of the integrated MFCM-Chip. (c) Scheme of the sample and reagent loading process. (d) Imaging of the nickel pattern arrays in eight parallel channels. (e) Schematic diagrams of the principle of multiplexed detection simultaneously in a MFCM-Chip. The scale bar is 500 μm.