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A fluoro-microbead guiding chip for simple and quantifiable immunoassay of cardiac troponin I (cTnI)

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ABSTRACT

We have developed a fluoro-microbead guiding chip (FMGC) to perform an optical immunoassay of cardiac troponin I (cTnI). The plasma marker protein cTnI is the currently preferred marker to use for a definitive diagnosis and prognosis of myocardial infarction. The FMGC has four immunoreaction regions on a silicon oxide substrate, with five gold patterns imprinted on each region for multiple simultaneous assays. The FMGC assay clearly distinguished immunospecific binding from nonspecific binding by comparing optical signals from inside and outside of the patterns. To detect cTnI, a sandwich immunoassay was performed using antibody-tagged fluoro-microbeads. The cTnI-specific capture antibody was conjugated to the FMGC surface by reaction with 3-3′-dithiobis-propionic acid N-hydroxysuccinimide ester to create a self-assembling antigen-sensing monolayer (DTSP SAM) on the chip. A sample containing cTnI was applied to the antigen-sensing monolayer and allowed to react. To generate a binding signal, a cTnI detection antibody-linked fluoro-microbead preparation was added. The cTnI concentration in a sample was determined by counting the number of biospecifically bound fluoro-microbeads on the corresponding five patterns on the FMGC. The optical signal showed a linear correlation with cTnI concentrations in plasma samples containing from 3.4 pM to 3.4 nM (0.1–100 ng/ml) cTnI. The sensitivity of cTnI detection could be increased by reducing the non-specific binding of the beads to the antigen-sensing surfaces of the chip. Optical detection and quantification of binding by fluorescence microscopy gave results that correlated well with results from a commercial ELISA for cTnI in human plasma. Based on these findings, we propose that the FMGC-based immunoassay system may be adapted to detect and quantify a variety of clinically important targets in human samples.

1. Introduction

Clinically important biomarkers present in blood, urine, and saliva at very low concentrations must be measured accurately to monitor states of health and disease (Giljohann and Mirkin, 2009; Zhang et al., 2007). The enzyme-linked immunosorbent assay (ELISA) provides adequate specificity and sensitivity for most markers (Mayilo et al., 2009). However, targets present at ng/ml concentrations may require a change in methodology. The limit of detection in the conventional ELISA is lies about between 0.3 ng/ml and 1 ng/ml. To increase ELISA sensitivity, a high enzyme-to-antibody ratio may be used to amplify the signal. For example, gold nanoparticles (AuNPs) (Ambrosi et al., 2010), polymer microspheres (Ke et al., 2010), or magnetic microbeads (Liu et al., 2010) may be used as scaffolds to stabilize the enzyme-antibody conjugate. Rissin et al. (2010) developed a single-molecule ELISA to detect prostate-specific antigen in human serum at picomolar concentrations.

In this study, we developed a fluoro-microbead guiding chip (FMGC)-based sandwich immunoassay for convenient and accurate biomarker quantification. The FMGC supported four immunoassay regions, each patterned with microchannels. Each immunosensing region contained five gold functional surfaces to perform five identical tests simultaneously and assess reproducibility (Fig. 1A).

The FMGC presents several advantages for use in an immunoassay system. First, the number of beads bound in each immunosensing region can be counted directly under a fluorescence microscope. This is both convenient and accurate. Second, the fluoro-microbeads attached on one immunosensing region can be viewed all at once, which allows the analyst to distinguish the specific immuno-binding (beads bound to the patterns) from non-specific binding (beads bound to the region outside of the patterns). Based on the fluoro-microbead counts inside and outside of the patterns and a simple numerical analysis, a biomarker may be simply and accurately quantified.

Cardiovascular diseases claim 17.1 million lives each year world-wide (http://www.who.int). Myocardial infarction (MI), one of the most severe adverse cardiac events, may cause irreversible tissue injury or necrosis in the myocardium. Although the MI

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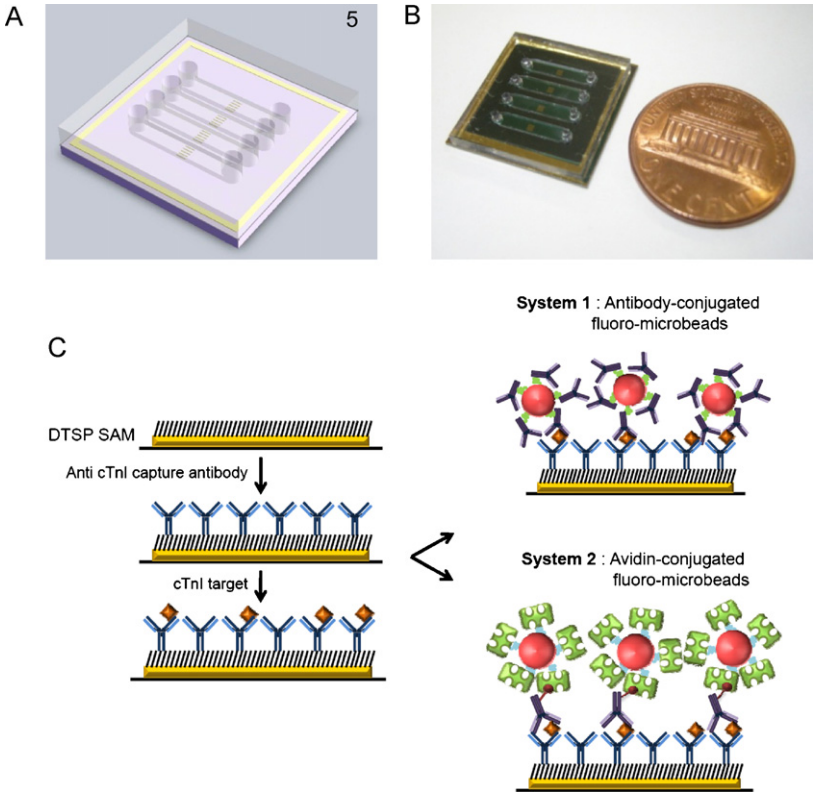

Fig. 1. (A) Design of the fluoro-microbead guiding chip (FMGC). (B) Photograph of the FMGC. (C) Schematic diagram of the sandwich immunoassay using antigen/antibody binding (System 1) and avidin/biotin affinity binding (System 2) on the FMGC. The avidin/biotin couple was used to enhance the signal.

diagnosis is based primarily on electrocardiograpy, only 57% of patients with acute myocardial infarction (AMI) exhibit electrocardiographic changes (McDonell et al., 2009; Suprun et al., 2010). A rapid and sensitive method to confirm AMI using specific cardiac markers is therefore desirable. The cardiac forms of troponin T (cTnT), troponin I (cTnI), creatine kinase-MB (CK-MB) and myoglobin provide valuable diagnostic markers for AMI and myocardial injury (Melanson and Tanasijevic, 2005; Piras and Reho, 2005; Wu et al., 2004). Among these markers, cTnI is highly specific to cardiac injury, showing little or no changes in patients with a skeletal muscle disease or trauma; creatine kinase (CK) and the CK-MB isoenzyme are less specific (Apple et al., 2004; Casals et al., 2007). No marker for AMI has yet shown greater specificity than cardiac troponin I (cTnI) (Panteghini et al., 2004). Blood levels of cTnI level in healthy humans are normally lower than 0.1 ng/ml. After the onset of AMI, however, the cTnI rises rapidly, within 3–6 h (Orbulescu et al., 2010; Wei et al., 2003), to levels ranging from 1 to 50 ng/ml, with wide variation. The cut-off cTnI concentration for an AMI diagnosis may be as low as 1 ng/ml, which corresponds to a desired detection limit of 0.1 ng/ml. This places cTnI among the biomarkers present in the lowest range of measurable concentrations (Ko et al., 2007). Following an MI, the cTnI remains elevated for 4–10 days, while most other cardiac markers decline more rapidly (Wu et al., 1999). cTnI may therefore be used to monitor patient status and predict outcome, as well as to diagnose AMI. In previous studies, several immunoassay techniques may be used to monitor cTnI (Bruls et al., 2009; Cho et al., 2009; Kiely et al., 2007; Mair et al., 1996; Todd et al., 2007).

In the present study, we devised an accurate and uncomplicated sandwich immunoassay mounted on an FMGC. The detection component was prepared by conjugation of cTnI antibody to fluoro-microbeads. The sensing surface was prepared by conjugation of cTnI capture antibody to DTSP-functionalized patterns on the chip. In the sandwich immunoassay, sample cTnI was allowed to bind to the chip surface. The fluoro-microbead conjugates were then reacted with the cTnI immobilized. The bound fluoro-microbead conjugates were counted directly using a conventional fluorescence microscope. The cTnI concentration was easily determined by counting the beads immobilized on immunosensing regions of the FMGC. Further, the optical signal from the region outside a pattern (the non-specific binding, or NSB signal) was used to control for the specificity of the immunosensing reaction. It was possible to accurately quantify cTnI through statistical analysis of data from a single test. Here we demonstrate that this method is suitable for cTnI biosensing using human blood samples.

2. Experimental

2.1. Chemicals and apparatus

3-3′-Dithiobis-propionic acid N-hydroxysuccinimide ester (DTSP), dimethyl sulfoxide (DMSO), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), avidin, Triton X-100, and sodium bicarbonate were purchased from Sigma–Aldrich, USA; cystamine, poly(amidoamine) generation 4 dendrimer (Dend), 4-formylphenylboronic acid (BA), ethanolamine and glutaraldehyde (GA), from Sigma–Aldrich; N-hydroxysulfosuccinimide sodium salt (sulfo-NHS), from Fluka; monoclonal antibodies to human cardiac troponin I (19C7 clone and 625 clone), from BioDesign; human cardiac troponin I (cTnI), skeletal isoform of troponin I (sTnI), cardiac troponin T (cTnT) and cardiac troponin C (cTnC), from Fitzgerald Industries International, USA; FluoSpheres® carboxylate-modified microspheres (excitation wavelength 540 nm and emission wavelength 560 nm, 2% solids, Ø = 0.2 μm), from Invitrogen; polyethylene glycol (PEG, MW = 3.4 kDa), from Polyscience Inc.; polydimethylsiloxane (PDMS) and its curing agent, from Dow Corning, USA; and a conventional cTnI ELISA kit, from AbFrontier, Korea.