A novel microfluidic device is developed which is, for the first time, able to detect protein and DNA simultaneously by using antibody and oligonucleotide biofunctionalized microbeads. The device consists of a gel pad array, which was used for the patterning of biofunctionalized microbeads, and a polydimethylsiloxane (PDMS) microfluidic module. By using the spatially encoded microbeads, the simultaneous detection of proteins and oligonucleotides was achieved on the array in a single microfluidic assay within 40 minutes.

**KEYWORDS:** Microbeads, Gel pad array, Spatial encoding, Immunoassay, Hybridization assay

**EXPERIMENTAL**

Preparation of the antibody and oligonucleotide coated microbeads

10 μm diameter streptavidin conjugated microbeads (Bangs Laboratories) were mixed with 10 times excess amount of either biotinylated monoclonal anti-β-hCG antibody (Arista Biologicals Inc.) or with biotinylated probe oligonucleotide (seq. 5’ biotin TCACATTITGGATAATCCCAACC, Proligo, Sigma), based on the binding capacity of the microbeads. After being washed, the antibody coated microbeads and the oligonucleotide coated microbeads was resuspended in 10 mM PBS buffer and TE buffer (10 mM Tris, 1 mM EDTA) respectively before storage at 4 °C.

Fabrication of the microfluidic device

The polyacrylamide gel pad array was fabricated on a glass slide by photopolymerization using a modified method from previous protocols [2, 3]. In brief, 10% polyacrylamide solution with 0.1% DMAP photoinitiator was injected into the gap between a Bind Silane (GE healthcare) pretreated glass slide and a photomask. The height of the gap was 12.5 μm which is defined by a mylar spacer. The solution was allowed to be photopolymerized in a UV crosslinker (UVC 500, GE Healthcare) for 5 min. The glass slide was then detached from the photomask, rinsed with distilled water and finally stored in a clean Petri dish.

A first batch of antibody coated microbeads (0.1 μl volume, ~200 beads/μl) was pipetted onto the gel pad array and dried for 3-5 min to achieve a stable immobilization. The second batch of oligonucleotide coated microbeads was then immobilized onto the array in the same manner. A picture was taken between the depositions of two batches of beads to record their spatial address. The microfluidic device was assembled by enclosing the array with a polydimethylsiloxane (PDMS) microchannel (2500 μm x 500 μm x 50 μm) which was fabricated using standard soft lithography [4].

Microfluidic assay for simultaneous detection of hCG and oligonucleotide

The microchannel was first blocked by introducing PBS-B-D-T buffer (10 mM PBS with 1 w/v% BSA, 1 mg/ml salmon sperm DNA and 0.05 v/v% Tween 20) at 2 μl/min for 5 min by using a syringe pump. PBS-B-D-T buffer spiked with 100 ng/ml of hCG and 100 nM of the Texas Red labeled target oligonucleotide (Seq. 5’ TxRed- GGTTGGGATTATCCAAAATGTGA, Proligo, Sigma) was then introduced into the channel at 2 μl/min for 10 min. After the channel was rinsed with PBS-B-D-T buffer at 5 μl/min for 5 min, 20 μg/ml Alexa Fluor 594 labeled polyclonal anti-hCG antibody was introduced at 2 μl/min for 10 min. Finally the channel was washed with PBS-B-D-T buffer at 5 ul/min for 5 min. The fluorescence image was then captured using a fluorescence microscope.
RESULTS AND DISCUSSION

One gel pad array unit contains 17 x 17 gel pillars (20 μm x 20 μm x 12.5 μm) with 10 μm gaps between pillars to allow the 10 μm microbeads to become trapped. The dimension of one unit of array was 500 μm x 500 μm which fitted well into the 510 μm wide PDMS microchannel (Figure 1).

The encoding process was done by recording the x,y coordinates of individual beads in each bead batch on the gel pad (Figure 2A and 2B) after every deposition step. The gaps between gel pillars enabled the stable immobilization of microbeads on the array, which eliminated the movement of microbeads during the encoding process. Thus, each microbead is encoded with a spatial code which remains unchanged during the whole encoding process as well as during bioassays. With the spatial codes, the bioprobes carried by individual microbead can be identified after the bioassay using a normal optical microscope.

By labeling the detection antibody and target oligonucleotide with a reporter dyes of similar excitation and emission spectra, the fluorescence signals from both immunoassay and hybridization assay can be obtained and analyzed in a single picture (Figure 2C). In this experiment, the signal from the immunoassay is much stronger than the signal from the hybridization assay, which could be due to the different kinetics of antibody-antigen binding and the binding of the two complementary oligonucleotides. Further optimization of the experimental conditions (e.g. the ion strength of the buffer, the flow speed) is required to enhance the performance of the hybridization assay.

With the spatial encoding method, by incorporating microbeads with different bioprobes, this device could be further used for multiplexing immunoassays, multiplexing DNA hybridization assays and even the simultaneous detection of different kinds of biomolecules (e.g. protein, DNA and metabolites) in real samples (e.g. serum, urine and environmental samples).

Figure 1. The microfluidic device for bead based bioassays (scale bar represents 100 μm)

Figure 2. Simultaneous detection of protein (hCG) and oligonucleotide in a single microfluidic assay (scale bars represent 50 μm).
CONCLUSION
The microfluidic device we propose here introduces a simple and effective method to simultaneously analyze proteins and DNA in the same sample. Using microbeads in a microchannel enables a fast binding kinetic between the bio-probe and the target analyte which results in shorter assay time (<40 min) compared to conventional methods such as ELISA (~4 h) and planar DNA microarray (~16 h). Furthermore, by using a spatial encoding method, multiplex bioassays can be easily performed with a single reporter dye and standard fluorescent microscope equipment.

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REFERENCES

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