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NOVEL QUANTITATIVE MACRO BIOMOLECULE ANALYSIS BASED ON A MICRO COULTER COUNTER

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ABSTRACT

We demonstrate quantitative biomolecule analysis using a micro coulter counter. Specific binding between antibody functionalized microparticles and target biomolecule cause large aggregates of microparticles. The micro coulter counter was employed to measure aggregation ratio, ratio of the aggregate counts to the total particle counts. Goat anti-rabbit IgG, used as a model biomarker, were tested in this paper. The experiment results showed that the aggregation ratio increases with the increasing biomolecule concentration, and the detectable concentration range from 16 to 160 ng/ml was achieved.

KEYWORDS: Coulter counter, Biosensing, Biomolecule, Quantitative analysis

INTRODUCTION

Biomarker detection represents an important task because biomarkers, as molecular indicators, are used to measure and evaluate biological states of the target subjects. Among various types of biomarkers, macromolecular biomarkers present in blood, such as glycoproteins, antibodies and enzymes, are of particular interest because the presence of various diseases is directly linked to abnormal concentrations of specific biomarkers in blood plasma or serum [1]. Thus, quantitative detection of biomarkers plays an important role in early diagnosing of many diseases, evaluating the extent of a disease, and monitoring the response to therapy. It is of the utmost importance to be able to detect and quantify biomarkers rapidly with portable, inexpensive devices. Immunoassay is a prevalent method for biomarker detection due to its high specificity. However, conventional immunoassays such as enzyme-linked immunosorbent assay (ELISA) require labeling antibodies, long assay time, and bulky, complicated detection instruments [2]. Here we present a label-free biomarker assay based on microparticle (MP)-biomarker immunoaggregation and a micro coulter counter to detect a target biomarker and measure the biomarker concentration.

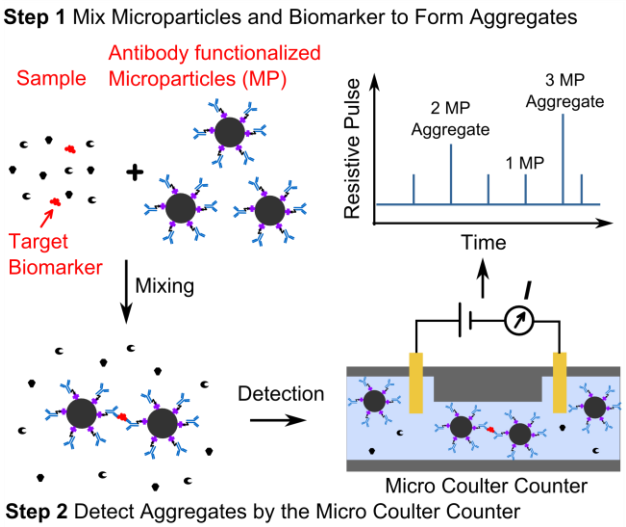

Figure 1: Illustration of the biomarker assay based on the immunoaggregation and a micro coulter counter.

As illustrated in Figure 1, the bioassay consists of two continuous, major steps: firstly, polyclonal antibody-functionalized microparticles are mixed with biomarkers in a sample, and form large-size MP-biomarker aggregates; secondly, the formed aggregates are detected by the micro coulter counter, which can accurately measure the size and count numbers of the aggregates [3].

EXPERIMENTAL

To prepare antibody factionalized MP, biotinylated rabbit anti-goat IgG antibodies were conjugated to 2.8 µm microparticles. Then, goat anti-rabbit IgG, used as a model biomarker, was mixed with MPs to form aggregates (see Figure 2 (a)). Biomarker concentration was varied from 16 to 320 ng/ml, while the MPs concentration (1.74×10^3 particle/µl) was kept constant for all samples. The micro coulter counter (see Figure 2 (b) and (c)) was used to measure the size and count the number of the aggregates/particles for each sample. The micro coulter counter was fabricated using the standard soft lithography method. It consists of a sensing channel with a width of 10 µm, height of 10 µm and a length of 30 µm to detect aggregates. Particle sizes were back calculated from pulse heights generated by passage of the particles through the sensing channel.

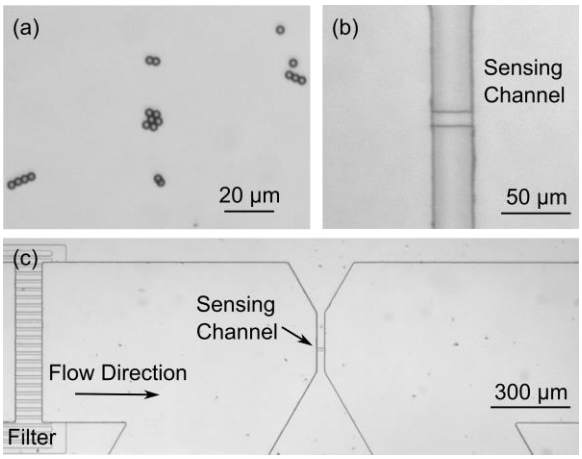

Figure 2: Microscopic picture of (a) formed aggregates, (b) and (c) the micro coulter counter channel.

RESULTS AND DISCUSSION

Figure 3 shows the measured aggregate size distribution and counts at a biomarker concentration of 160 ng/ml. The aggregation ratio of the aggregates counts to total particles/aggregates counts, at each biomarker concentration was analyzed and plotted in Figure 4.

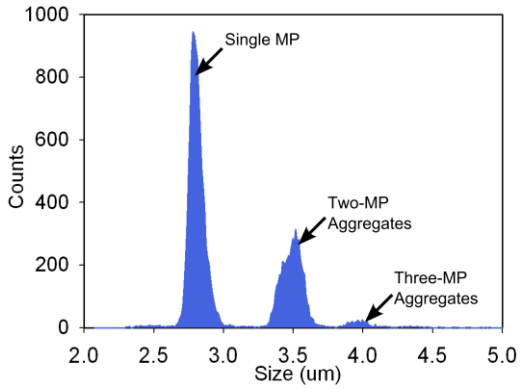

Figure 3: Measured aggregation size distribution and counts at a biomarker concentration of 160 ng/ml. The aggregation ratio, ratio of aggregates counts to total particles counts, is 28.1%.

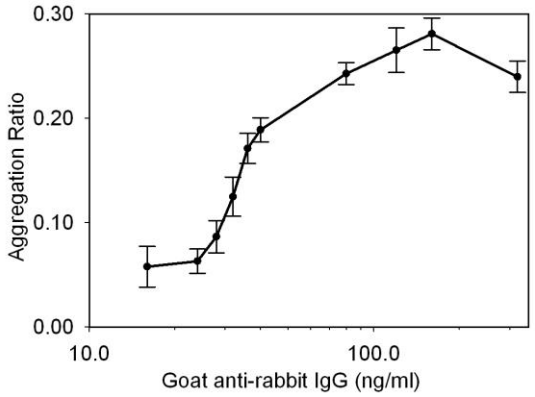

Figure 4: Measured aggregation ratio as a function of goat anti-Rabbit IgG concentration. The goat anti-Rabbit IgG concentration was varied from 16 to 320 ng/ml.

As shown in Figure 4, as the biomarker concentration was increase from 16 to 320 ng/ml, the aggregation ratio was increased from 5.8% to 28.1%. Note that at a high biomarker concentration >320 ng/ml, biomarkers occupy all the binding sites of the antibody for forming the aggregates; hence the aggregation ratio starts to drop. The above testing results have demonstrated the sensing principle and the feasibility of microparticle-biomarker immunoaggregation based micro coulter counter for sensitive biomarker detection.

CONCLUSION

We demonstrated a quantitative biomarker assay based on immunoaggregation and a micro coulter counter. Goat anti-Rabbit IgG, as a biomarker, was tested as a model biomarker. The detection range from 16 to 160 ng/ml was achieved. With simple structure, label free and ability to quantitatively measure biomolecule, this device is a promising tool for both clinical diagnostics and bio-analysis research.

ACKNOWLEDGEMENTS

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