Proceedings of the 2003 IEEE International Frequency Control Symposium and PDA Exhibition Jointly with the 17th European Frequency and Time Forum

RAPID DETECTION OF FIBRINOGEN AND FIBRIN DEGRADATION PRODUCTS BY LATEX PIEZOELECTRIC IMMUNOASSAY

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ABSTRACT

It was developed a conventional immunosensor for fibrinogen and fibrin degradation products (FDP) to combine a quartz crystal microbalance (QCM) with the agglutination reaction of immunized latex beads. We successfully measured FDP concentration of in human serum within 10 min by QCM method. The detection range of QCM immunosensor is covered with screening concentration of FDP in serum (less than 10 μg/ml of FDP). The time course of latex agglutination obtained from QCM immunosensor is synchronized to that of latex photometric immunoassay. Frequency shift on immunoreaction explains the increased adsorption amount of agglutinated latex on QCM.

INTRODUCTION

Among various kinds of medical diagnosis, immunoassay is particularly useful for conventional diagnosis. Elevated concentrations of fibrinogen and fibrin degradation products (FDP) have been found in serum of patients with venous thrombo-embolism and disseminated intravascular coagulation (DIC), but also in the case of trauma, surgical procedures, infections, malignancies, and sickle-cell anemia.

Rapid detection of FDP assays is valuable to evaluate response to therapy and is available for identifying risk control for patients. Latex agglutination assay is regarded as a validation test for doubtful cases. However, latex agglutination assay conduct requires highly trained examiners. Latex photometric immunoassay for FDP developed using latex particle immobilized with FDP antibody has been used to solve such problems [1]. When an antigen is added to the suspension of antibody-immobilized latex beads, immunoreaction induces bead agglutination. These reactions are measured by change in light absorbance. The automatic analyzer of FDP is a sensitive and precise technique yielding reproducible results, but is a very expensive apparatus. Therefore, a cost-effective, convenient and rapid detection method without a labeled reagent for FDP detection is needed.

A piezoelectric quartz crystal can be used effectively as a microbalance. Adsorption of material on the crystal surface changes its oscillation frequency. Adsorption of about 1 ng of a material causes a frequency shift of 1 Hz when an AT-cut quartz crystal of 9MHz is used [2]. Many studies have used a quartz crystal microbalance (QCM) as a transducer of immunosensor coated with antibody or antigen on the electrode surface [3-4]. The QCM is used as a viscosity sensor for clinical diagnosis

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[5]. We have developed a new immunoassay method which combines QCM with antibody or antigen-immobilized latex beads. This method is called latex piezoelectric immunoassay (LPEIA) [6]. Using this method, we succeeded in measuring concentration of C-reactive protein (CRP), antistreptolysin O (ASO), rheumatoid factor (RF), and *Treponema pallidum* (TP) in serum [7-9]. In this paper, we focus to construct the conventional detection method of FDP using LPEIA. It is expected that QCM method could be applied to clinical analysis of FDP as a rapid and on-site immunosensor.

MATERIALS AND METHOD

Suspension of the latex immobilized anti-fibrinogen degradation products antibody (Seratestam FDP, ST-8000) and FDP standard serum (Seratestam S FDP: STS-8000, 26.3 μg/ml of FDP in serum) were obtained from Kainos Laboratories Inc. (Tokyo, Japan). FDP sample solutions were prepared by dilution of a standard serum with saline solution to 2.63, 3.76, 5.26, 8.77, and 13.15 μg/ml. Figure 1 indicated schematic diagram for measurement system of latex beads agglutination using QCM device. When FDP is added to a suspension of anti-FDP antibody immobilized latex beads, immunoreaction induces bead agglutination. These immunoreactions are measured by QCM frequency change. The QCM used gold electrodes on both surfaces was an AT-cut of 9 MHz ($8 \times 8 \times 0.15$ mm^3) purchased from Nihon Denpa Kogyo Co., Ltd. (Sayama, Japan). To achieve stable oscillation in latex suspension, one side of the crystal was sealed with silicon sealant and a quartz crystal plate.

The entire apparatus for LPEIA was maintained at 20 ± 0.1°C in an air chamber. The sealed quartz crystal was set in a small cuvette made of polymethylmethacylate ($10 \times 10 \times 15$ mm^3). Then, 100 μl of latex suspension was added to 1,200 μl of phosphate buffer (pH 7.0 and 10 mM). The phosphate buffer was formed by mixing Na_2HPO_4 and KH_2PO_4 solutions. The suspension was stirred by a magnetic stirrer. The quartz crystal oscillation frequency stabilized about 30 min after being immersed in the latex solution. When 20 μL of FDP serum was added, frequency shift was observed and maintained 60 min for each solution.

Latex photometric immunoassay was performed with a MultiSpec-1500 spectrophotometer (Shimadzu Co., Ltd., Kyoto, Japan). The time course of absorbance change on latex suspension was measured at 570 nm. The entire apparatus for LPIA was maintained at 20 ± 0.1°C in an air chamber. The cuvette made of polymethylmethacylate ($10 \times 10 \times 30$ mm^3) was set in a photometer. Then, 150 μl of latex suspension was added to 1,800 μl of phosphate buffer (pH 7.0 and 10 mM). The suspension was stirred by a magnetic stirrer. Steady absorbance was obtained about 30 min after immersion in the latex solution. When 30 μL of FDP serum was added, the absorbance change was observed and maintained 60 min for each.

RESULTS AND DISCUSSION

Figure 2 shows the oscillation frequency shift at 10 and 60 min as a function of FDP concentration. A linear relationship was observed according to FDP concentration. Respective regression lines were: 60 min, ΔF_{60} = 8.489 [FDP] + 11.58 for FDP (correlation coefficient, r = 0.999) and 10 min, ΔF_{10} = 4.411 [FDP] + 0.844 for FDP (correlation coefficient, r = 0.993). Frequency shift at 60 min was higher than 10 min, suggesting that detection time of LPEIA for FDP can be reduced to only 10 min. To evaluate suitability of QCM method, correlations of frequency shift and absorbance change of latex suspension are shown in Fig. 3. Respective regression lined were: 60 min, ΔF_{60} = 170.1 [ΔAbs_{60}] + 11.76 for FDP (correlation coefficient, r = 0.997); 30 min, ΔF_{30} = 158.5 [ΔAbs_{30}] + 11.43 for FDP (correlation coefficient, r = 0.994); and 10 min, ΔF_{10} = 395.4 [ΔAbs_{10}] + 2.220 for FDP (correlation coefficient, r = 0.993). Correlation between LPIA and LPEIA is extremely good.

In clinical analysis, the DIC stage is determined by

monitoring FDP values in serum. FDP assay kits, which are usually used for testing purposes, have a detection range of below 10 μg/ml. The FDP value in a healthy person's serum shows the value of less than 10 μg/ml. Thus, this LPEIA sensor is sensitive enough for biological investigation of DIC and can be carried out with human serum. Consequently, this QCM sensor offers several advantages over usual tests; it is suitable for emergency cases, since obtaining serum completely devolved in fibrinogen, especially for patients under heparin therapy, is time consuming.

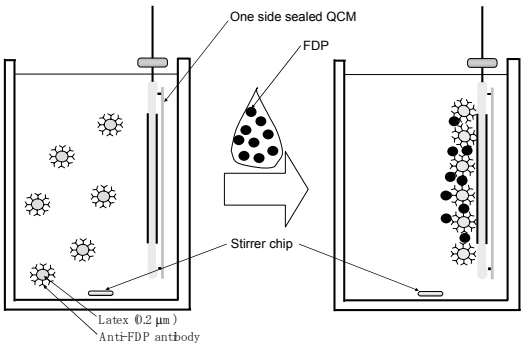

Figure 1. Schematic diagram for Agglutination reaction of immunized latex beads using QCM technique.

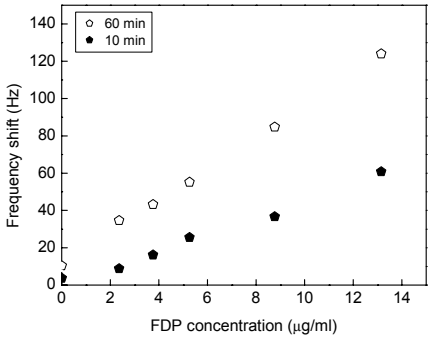

Figure 2. Relationship between frequency shift and FDP concentrations.

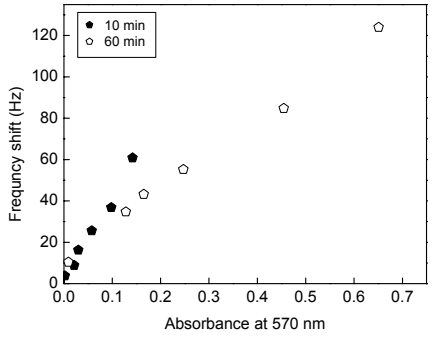

Figure 3. Relationship between frequency shift and absorbance change.

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