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Short Communication

Inkjet monitoring technique with quartz crystal microbalance (QCM) sensor for highly reproducible antibody immobilization

Y. Fuchiwaki [a,b,*], Y. Yabe [b], Y. Adachi [b], M. Tanaka [a], K. Abe [a], M. Kataoka [a], T. Ooie [a]

[a] Health Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), 2217-14, Hayashi-cho, Takamatsu, Kagawa 761-0395, Japan
[b] Development Division, Cluster Technology Co., LTD., 5-28, 4-chome, Shibukawa-cho, Higashi-Osaka 577-0836, Japan

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ABSTRACT

We demonstrate that a calibrated amount of a biomolecule can be microdeposited using a commercially available quartz crystal microbalance (QCM) and a piezoelectric inkjet head with a flattened surface surrounding the ejection hole. Covering the QCM with a stainless-steel cover perforated with a precisely-machined hole both significantly decreased background noise due to external sources such as air flow and temperature fluctuations and allowed accurate droplet ejection at a constant temperature. Anti-c reactive protein antibody solution (anti-CRP; 1.0 mg/ml) was continuously ejected onto a microchannel target using a piezoelectric injector; its microdeposition was monitored shot-by-shot using a 30 MHz QCM. The dispersion of the QCM frequency change per shot decreased from 7.87 to 1.01 Hz when a perforated stainless-steel cover was installed. The primary antibody was precisely deposited on the target with high reproducibility. The variation in luminescence measured by enzyme-linked immunosorbant assay (ELISA) decreased from 9.67% to 1.16% when calibrating the amount of primary antibody deposition with this developed technique.

1. Introduction

Patterning substrates with biological reagents using inkjet ejection is a critical component of microbioanalysis systems. Since the printing of 3D structures by inkjet microdeposition was first demonstrated, inkjet printers have been used to deposit micro amounts of biomedical fluids [1–7]. Picoliter quantities can be deposited and patterned in a single process with drop-on-demand (DOD) control. The use of DOD inkjet printing technology has considerably reduced the total number of processes required for direct writing [8–13]. Furthermore, although etching and lithography are conventionally used to form patterns on substrates, inkjet printing can directly produce patterns or 3D structures without using lithography or chemical etching. Inkjet technology can be used for a very wide range of micropatterning and microdeposition applications, and has recently been applied to on-demand manufacturing and microfluidic technologies.

The method employed for inkjet ejection directly affects the final print quality. Either continuous or DOD piezoelectric and/or thermal techniques can be used to form and eject droplets; the technique used determines the droplet size and properties. As a practical inkjet monitoring application, some published literatures discuss with piezoelectric self-sensing [14–17] and piezoelectric driven MEMS print-head [18].

We recently fabricated an immunoassay microchip for enzyme-linked immunosorbent assay (ELISA) using a piezoelectric inkjet printing system manufactured by Cluster Technology Co., Ltd. (Osaka, Japan) [8,9,19,20]. This microchip was used to construct a sandwich ELISA for the quantitative analysis of biomarkers in the blood, and piezoelectric inkjet printing was used to deposit and fix a primary antibody on the chip's microchannel surface.

Despite these advances, it remains difficult to deposit antibodies in specific locations with sufficiently high reproducibility. In particular, a high concentration of antibody destabilizes the ejection spray from the inkjet printing head because the viscosity of a droplet generally increases with increasing antibody concentration. Since the primary antibody for ELISA is present in solution at a comparatively high concentration, it is important to examine and calibrate the ejection stability of the printing head.

There are essentially two methods for determining ejection stability and deposition: monitoring the droplet trajectory, and monitoring droplet impact. The droplet trajectory is monitored using synchronized stroboscopic illumination. This method can measure the droplet velocity in real time and estimate the

* Corresponding author at: Health Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), 2217-14, Hayashi-cho, Takamatsu, Kagawa 761-0395, Japan. Tel.: +81 87 869 4201; fax: +81 87 869 4178.
E-mail address: yu-fuchiwaki@aist.go.jp (Y. Fuchiwaki).

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approximate volume of the droplet, but it cannot accurately measure the amount of liquid deposited by the inkjet printer.

Monitoring the droplet impact can measure the amount of liquid deposited by using a labeled biomolecule, a sophisticated camera, and a label-free detection device such as a quartz crystal microbalance (QCM). Several reports have described the use of specifically labeled materials to construct 3D patterns in a layer-by-layer manner [1,21,22]. Although fluorescently-labeled reagents are effective for estimating the amount of liquid deposited, light scattering may prevent accurate mass determinations; furthermore, it is necessary to prepare and label fluorescent compounds. In contrast, a QCM sensor can measure the mass per unit area without the need to label the materials. However, QCM sensors are generally used in a precisely-controlled environment, making it difficult to monitor deposition in air. The same is also true of other label-free detection methods such as ellipsometry, surface plasmon resonance spectroscopy, and dual polarization interferometry.

Against this background, we seek to accurately calibrate the deposition of antibodies by using a commercially available QCM sensor and inkjet head with a flattened surface around the ejection hole at atmospheric pressure and room temperature. The aim of the present study is to continuously detect ejection on a shot-by-shot basis using a piezoelectric injector and to develop an easy-to-use technique for highly reproducible antibody immobilization.

2. Experimental

2.1. Reagents and equipment

A human C-reactive protein (CRP) EIA Kit was purchased from CycLex Co. Ltd. (Nagano, Japan). CRP is a widely-used biomarker for evaluating inflammation; consequently, quantitation of CRP levels is useful for determining the progress of a disease or the effectiveness of treatments.

A QCM sensor purchased from Tamadevice Co. Ltd. (Tokyo, Japan) was used to estimate the amount of anti-CRP deposited. The frequency counter in the QCM was purchased from Turtle Industry Co., Ltd. (TUSB-S03CNBZ, Ibragi, Japan). A QCM electrode was fabricated by depositing gold on both sides of a quartz crystal with a chromium underlayer. The resonance frequency was 30 MHz and the electrode diameter was 5.0 mm. The resonators were connected by a silver conducting paste via wires to a BNC connector. The mass detection sensitivity of the QCM sensor is defined in terms of the mass of the detected target molecules per unit area of the gold surface for a given change in the resonance frequency of the QCM. A higher resonance frequency will lead to greater sensitivity of the QCM. In this study, a highly sensitive commercially available QCM with a resonance frequency of 30 MHz was used. Although a sensitive QCM sensor can accurately detect the deposition amount, the background signal is greatly amplified by external influences such as temperature fluctuations, vibrations, air flow, and other factors. Therefore, in this study, we seek to detect the deposition amount in the absence of external fluctuations.

The piezoelectric inkjet printing system used was manufactured by Cluster Technology Co., Ltd. [23,24]. The printing head (PulseInjector®) is a piezo-driven DOD head made from epoxy resin composite with a flat surface surrounding the ejection hole. By combining the PulseInjector® and a dedicated driving unit (WaveBuilder®), it is possible to freely adjust the ejection drive waveform, the ejection rate, and the driving voltage, enabling droplets to be ejected on demand in picoliter quantities. The experimental system used for inkjet printing (DeskViewer™) can monitor the droplet trajectory and impact in real time for evaluating droplet ejection.

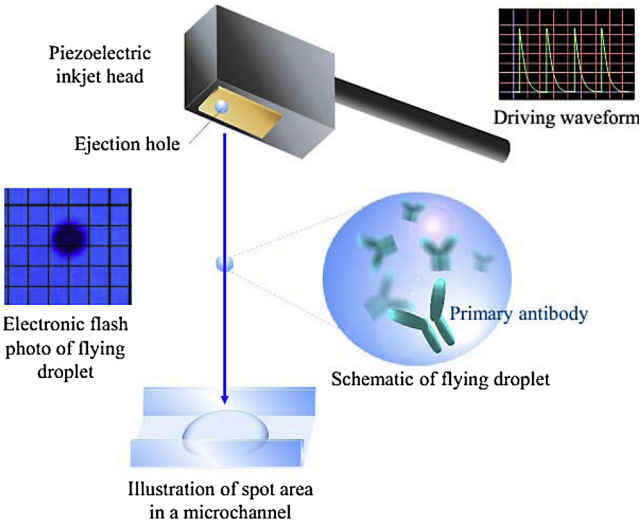

Fig. 1. Schematic illustration of inkjet microdeposition of primary antibodies on the microchannel surface.

Anti-CRP aqueous solutions with concentrations between 0.3 and 1.0 mg/ml were used to evaluate the shot ejection system. The PulseInjector® had a 25-μm-diameter hole. The amount ejected per shot was estimated to be approximately 30 pl, judged from analysis of the droplet trajectories.

3. Results and discussion

3.1. Miniaturization of frequency fluctuation of the QCM sensor with a perforated stainless-steel cover

Practical immunoassay microchips for sensitive and accurate ELISA require high-precision determination of primary antibody deposition (Fig. 1) [8,9]. To our knowledge, there have been no reports of a continuous, shot-by-shot technique for monitoring the deposition of a high-viscosity liquid containing a high concentration (up to 1.0 mg/ml) of antibody using only a readily available QCM sensor. The frequency of a QCM sensor is greatly influenced by background noise from external factors such as air flow and temperature fluctuations. The stainless-steel cover supplied with the commercially available QCM sensor fit well over the PulseInjector® and so was used to reduce the background noise. The resonance frequency of the QCM sensor was stable when the stainless-steel cover was securely fit over the sensor and the temperature was constant. If a hole is precisely machined into the stainless-steel cover to allow droplet ejection, and the cover closely contacts the PulseInjector®, then the reduction in frequency fluctuation should be similar to that obtained prior to machining the hole.

To test this hypothesis, we constructed a simple, portable and inexpensive QCM sensor unit with a hole in the stainless-steel cover (Fig. 2a). The resonance frequency was measured to evaluate whether the perforated cover effectively reduces external background noise (Fig. 2b).

By employing some simplifying assumptions, the frequency fluctuation of the QCM sensor was quantified and precisely correlated with the mass change using the Sauerbrey equation. For the QCM sensor with a resonance frequency of 30 MHz, a frequency change of 1 Hz is estimated to be equivalent to 20 pg. The laboratory temperature was approximately 23 °C.

The results revealed that the frequency fluctuated considerably without the perforated cover in place, whereas when the perforated cover was installed, the frequency stabilized (Fig. 3a). The average