Controlling Antibody Immobilization by Laser Micro-processing

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Understanding the state of an individual's health is a complex procedure that requires simultaneous measurement of several markers. The enzyme-linked immunoSorbent assay (ELISA) method generally used to perform blood tests requires many samples, and antigen-antibody reactions take a long time. We have developed a plastic chip that simultaneously achieves sandwich ELISA protocols of multiple targets in one channel. This chip leads to a reduction in both the sample quantity and the in reaction time.

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1. Introduction

ELISA is a method for detecting antigens of samples by using antibodies as probes. It has also been reported that laser surface treatment improved immobilization ability of an antibody better than chemical surface treatment [1]. However, the comparison of chemical surface treatment and laser surface treatment shows that although immobilization increases with laser surface treatment, it is unstable.

Our developed microchip has detection points with immobilized antibody dots. To develop this chip, antibody solutions were ejected by an inkjet device into a micro-channel surface. However, when detection points were created on the micro-channel surface, it was difficult to control the shape of the immobilized area formed by this process. Furthermore, the detection points that form uneven shapes affect the stability of the immobilization capacity.

In this study, we report the increase in the stability of the antibody capacity when two processes are performed in addition to surface treatment: the first process involves wet etching with an organic solvent on a PMMA substrate [2, 3] and the second process involves forming a hollow structure on a channel with laser micro-processing.

2. Experimental

An immunoassay chip was produced using laser irradiation and was processed under two wash conditions: water washing and wet etching. Antigen detection experiments were performed under both conditions and the results were compared. In addition, we used laser to form small hollow depressions in the channel at the detection points and the effects of the shape were compared.

2.1 PMMA Substrate

In this experiment, we used a PMMA substrate, obtained by injection molding. Fig. 1 shows the structure of the microchip substrate, which has four channels each of depth 100 μm, width 300 μm, and length 60 mm. Sample inlets and outlets of 1 mm diameter are present at both ends of the channels. Reagents for antigen-antibody reactions and chemiluminescence were injected in the channels formed by covering the grooves from the inlets and exhausted from the outlets.

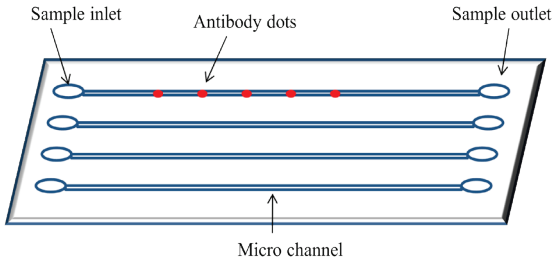

Fig. 1 Structure of the microchip substrate for antibody immobilization.

2.2 UV Laser Processing

An ArF excimer laser (193 nm), EXL-210S (JAPAN STORAGE BATTERY CO.LTD.), was used for processing. The specifications of the laser oscillator are shown in Table 1.

Table 1 Specifications of the laser.

Max. pulse energy	300mJ
Max. power	30W
Max. repetition rate	200pps
Pulse width	14ns

The schematic of the laser processing system used in this study is shown in Fig. 2. The magnification is 1/10 and

masks with oblong (4.5 mm × 10 mm), and circular (diameter 2.8 mm) aperture were used.

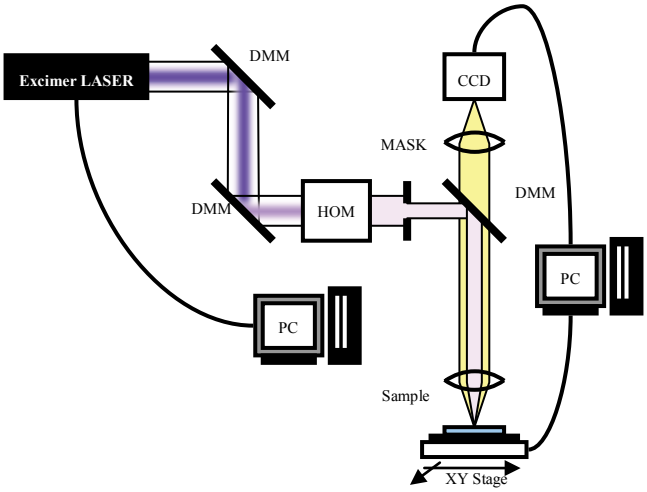

Fig. 2 Schematic of the ArF excimer laser and optics used for PMMA micro-processing

2.3 GG Solution Etching

To perform surface treatment of the laser-irradiated area on the substrate, we used an organic solvent mixture known as "GG developer [2] for washing and wet etching. The contents of the GG solution are shown in Table 2.

Table 2 Contents of GG solution

Developer solution

2-(2-n-butoxyethoxi)ethanol	60 wt%
Morpholine	20 wt%
2-aminoethanol	5 wt%
Water	15 %

Stopper solution

2-(2-n-butoxyethoxi)ethanol	80 wt%
Water	20 %

After laser irradiation, the sample was first placed in the GG developer and then the stopper solution with ultrasonic agitation for 1 min in each solution. Finally, the sample was washed with deionized water and air dried.

2.4 Antibody Solution

Procollagen type I C-peptide (P1CP) antibody solutions were ejected by an inkjet device into the micro-channel. P1CP is used as a bone metabolism marker.

2.5 Chemiluminescence Measurement

The procedure for sample detection from microchip fabrication is as follows.

(1) The chip surface is treated by laser processing.
(2) During the creation of detection spots, the antibody solution is injected into the channels by using the inkjet device.
(3) The micro channels are covered by the PMMA film with an adhesive layer.

The sample and the reagent are placed in the channel inlets and the biomarkers are detected by the antigen-antibody reaction. In this experiment, we used a detection method called the sandwich ELISA protocol with the following procedure.

(1) Leave the chip at room temperature after the antibody solution is ejected.
(2) Fill the channels with the blocking liquid and leave for one hour.
(3) Inject and exhaust the cleaning solution three times.
(4) Fill the channels with the compound liquid consisting of the antigen and the second antibody (labeled as a peroxidase) and leave for 30 min.
(5) Inject and exhaust the cleaning solution five times.
(6) Fill the channels with a chemiluminescence substrate.
(7) Measure the signal intensity with a CCD camera.

3. Results and Discussion

In this section, we describe the results of the P1CP antigen detection experiments performed in laser-irradiated PMMA by water washing and wet etching. In addition, we describe the results of the P1CP antigen both with and without the formation of a hollow structure.

3.1 Laser Irradiation of PMMA Substrates

Fig. 3 shows the laser-irradiated region in the bottom of the channel. The laser-irradiation condition is 30 shots at a fluence of 0.03 J/cm^2. Particles observed on the surface of the substrate were generated during laser irradiation. These are considered to be solidified PMMA vaporized by laser irradiation.

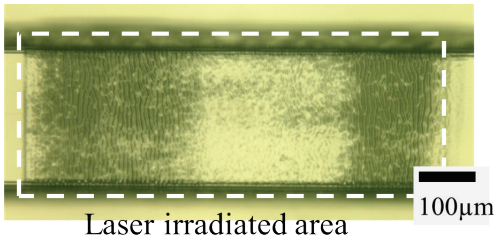

Fig. 3 Optical microscopic image of a laser-irradiated area

Fig. 4 shows a comparison of the emission intensities in the untreated and laser-irradiated areas. For each condition, three detection points were prepared, and the emission intensities were measured. The total volume of the solution in each detection point was 4.58 nl. The substrate surface that was irradiated by UV laser showed an-increased emission intensity. However, the instability of the emission intensity was also apparent. We believe that the particles on the laser-irradiated area affected antibody immobilization, resulting in the instability of the emission intensity.

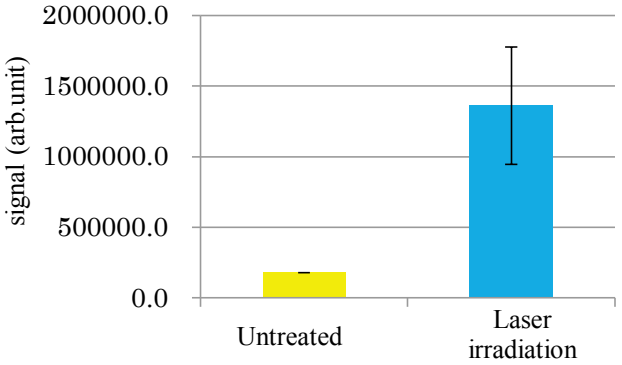

Fig. 4 Comparison of Signal intensities and standard deviations in the untreated, and laser irradiated areas.

3.2 Effect of Wet Etching

Fig. 5 illustrates the laser irradiated area in case of wet etching. In this case, particles observed on the surface of the substrate were removed.

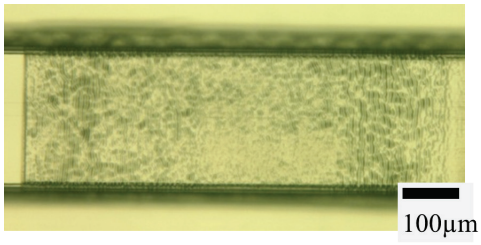

Fig. 5 Optical microscopic image of the laser-irradiated area with wet etching.

Fig. 6 shows a comparison of the emission intensities in laser-irradiated PMMA with water washing and wet etching. For each condition, three detection points were prepared, and the emission intensities were measured. The total volume of the solution in each detection point was 4.58 nl. It is observed that in both cases, there was no decrease in the emission intensities and there was a significant increase in the stability of the emission intensities. As a result, the debris removed by the etching process depended on the variation in the antibody immobilization ability. We believe that this was because of the variation in the emission intensities of the detection points.

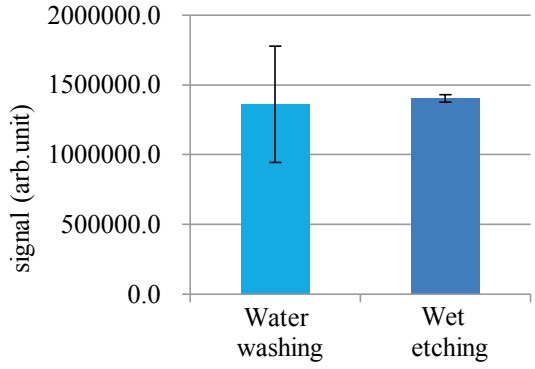

Fig. 6 Comparison of Signal intensities and standard deviations of the laser-irradiated area with water washing and wet etching.

3.3 Effect of Forming Detection Point

Problems were encountered when detection points were created on the micro-channel surface by the inkjet device. Fig. 7(a) shows the problems regarding the formation of uneven shapes with the antibody detection solution. Fig. 7 (b) shows the solution attached to the wall of the channel. The antibody detection solution forms uneven shapes because of the surface conditions of the substrate, such as contact angle, species, and concentration of the solution.

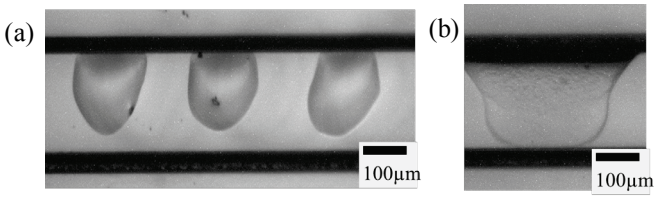

Fig. 7 Optical microscopic image of the antibody detection solution (a) forming uneven shapes and (b) attached to the wall of the channel.

We verified whether the form of the detection point was linked to the variation in the emission intensity. Fig. 8 shows the detection points under various conditions used for comparison. The total volume of the solution in each detection point was 2.65 nl. Fig. 8(a) shows the detection points attached to the wall of the channel. Fig. 8(b) shows the detection points that form even shapes. For each condition, three detection points were prepared and the emission intensities were measured.

Fig. 9 shows chemiluminescence for the conditions shown in Fig 8. It is observed that the luminescence intensity of the detection point attached to the wall is strong. This implies that the area of the detection point becomes large in proportion to the immobilized surface area when the antibody solution is attached to the wall.

Fig. 10 shows the comparison of the emission intensities for the two conditions shown Fig. 8. Because of the high emission intensities of the detection points attached to the wall, we observe the variations in the emission intensities.

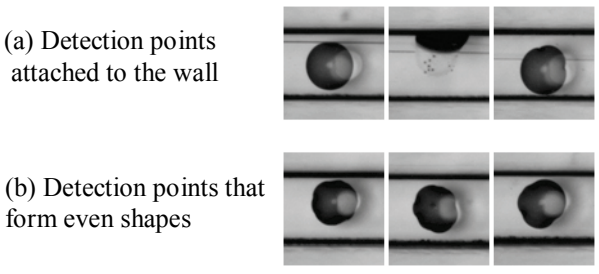

Fig. 8 Optical microscopic images of the antibody detection solution (a) detection points attached to the wall (b) detection points that form even shapes.

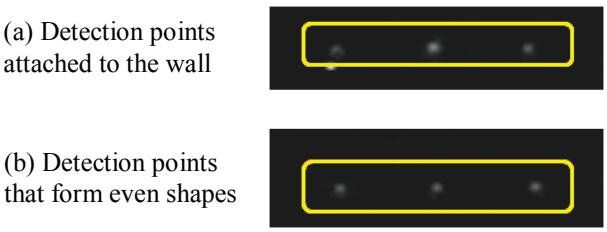

Fig. 9 An image of chemiluminescence from detection dots with a P1CP antigen solution (a) detection points attached to the wall (b) detection points that form even shapes. The exposure time was 15 min.

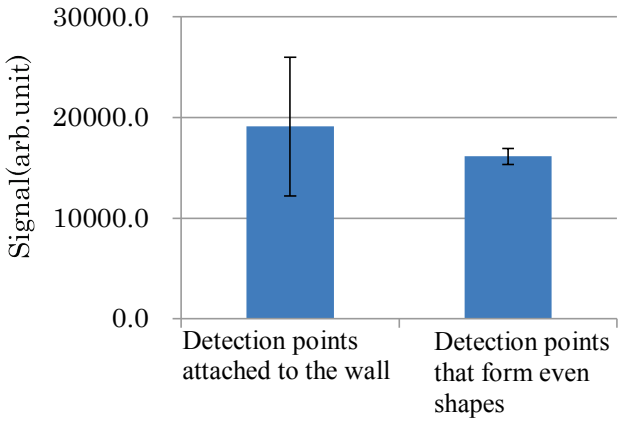

Fig. 10 Comparison of signal intensities and standard deviations of detection points attached to the wall and detection points that form even shapes.

3.4 Effect of Hollow Structure

To control the shape and position formed by the antibody detection solution, we used laser micro-processing, as illustrated in Fig. 11 (a). Using laser, we formed a small hollow structure in the channel with a depth of 30 μm, and a diameter of 260 μm at the detection points. The laser processing condition was 80 shots at a fluence of 0.05 J/cm^2.

Fig. 11 (b) shows the test hollow structure and the results when P1CP antibody solutions were injected into it. It is observed that the antibody solutions control the shape, position, and area of the detection points, and prevent the solution from being attached to the channel wall.

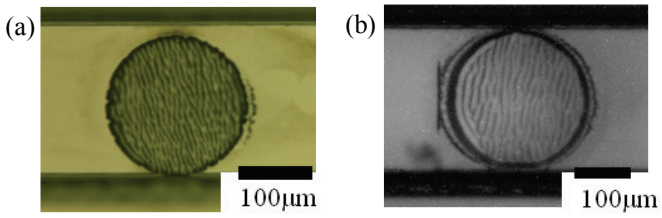

Fig.11 Optical microscopic image showing the processed hollow structure in (a) a micro-channel and (b) the controlled shape and position of the antibody detection solution formed by the inkjet device.

Fig.12 shows the comparison of the emission intensities in the laser-irradiated area and the hollow structure by the laser process. For each condition, three detection points were prepared, and the emission intensities were measured. The total volume of the solution in each detection point was 5.3 nl. It was observed that in both cases, there was no decrease in the emission intensities and there was a significant increase in the stability of the emission intensities. As a result, we can confirm that this hollow structure is not an obstacle to detection.

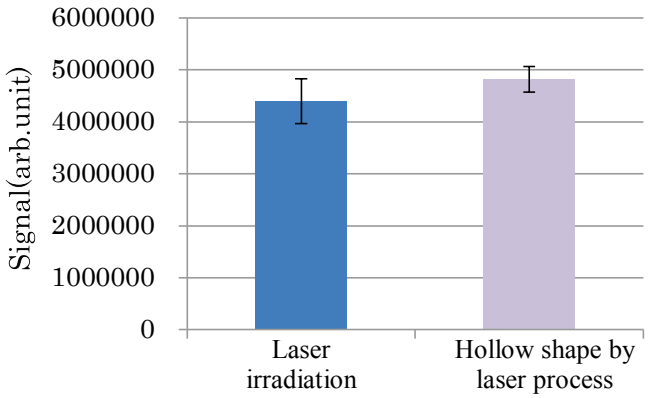

Fig. 12 Comparison of signal intensities and standard deviations of the laser-irradiated area and the hollow structure obtained by the laser process.

4. Conclusions

The processing method that combines laser irradiation and etching resulted in improvement and stabilization of antibody immobilization. Furthermore, the hollow structure formed in the channel by laser processing maintains the antibody immobilization capacity for controlling the shape, position, and area of the detection point. It also prevents the solution from becoming attached to the channel wall. This method can therefore result in the stabilization of antibody immobilization.

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