

Technical note

Photoimmobilized array of panel cells for assay of antibodies

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Abstract

Antibodies in blood are checked with panel blood cells before blood transfusion. In this investigation, for the first time, a panel cell-microarray was prepared by using a photoimmobilization method. Different types of red blood cells were microarrayed on a plate. A water-soluble photoreactive polymer as a matrix was synthesized by the coupling reaction of azidoaniline with poly(2-methacryloyloxyethylphosphorylcholine-co-methacrylic acid). The polymer was mixed with cells and the mixtures were microspotted on substrate and photoirradiated after drying in air. For the antibody assay, monoclonal antibodies or human serum was added to the cell-arrayed plate and adsorbed antibodies were detected by horseradish peroxidase-labeled secondary antibody, which recognized the adsorbed antibodies. Antibodies specifically adsorbed on the immobilized cells as expected. The aggregation method has been available for this type of assay, but extensive experience was needed to apply it correctly. The method using a cell array will be useful for antibody detection.

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1. Introduction

Microarray technology has become a crucial tool for large-scale and high-throughput biological science and technology. It allows fast, easy, and parallel detection of thousands of addressable elements in a single experiment under exactly the same conditions [1–3]. DNA microarray approaches have demonstrated a rapid and economic way to interpret gene functions [4,5]. In recent years, there have been considerable achievements in preparing small-molecule arrays [6,7], peptide arrays [8–11], protein arrays [12–15], polysaccharide arrays [16–18], antigen arrays [19–21], antibody arrays [22–27], and tissue arrays [28,29].

Although the preparation of the microarray is important, no universal immobilization method has been developed. Noncovalent immobilization (physical adsorp-

tion) or standard chemical coupling reactions (using amino or carboxyl groups in the materials) were usually used. However, the former is not suitable for stable immobilization and the latter is limited by the structure of the immobilized materials. Therefore, Ito et al. developed a photoimmobilization method for the preparation of a microarray chip [30]. Using this method, any material can be immobilized on a substrate by the use of radical reactions. Ito et al. microarrayed proteins using photoreactive polymers as the matrix on a tissue culture polystyrene plate [30,31]. In the present study, cells were microarrayed using this method. Although a microarray of cultured cell lines for antibody detection of cell surface proteins has been reported by Schwenk et al. [32], the use of a cell microarray for clinical analysis has not been reported.

Here, the assay of antibodies in blood was chosen as the target of the cell array. Usually, A-type blood does not have anti-A antibody. However, irregular antibodies are sometimes produced by previous transfusion or pregnancy. Blood that contains irregular antibodies cannot be used for

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transfusion, because they aggregate with the patients' cells. Therefore, the presence of irregular antibodies is usually checked before transfusion. However, as the check is performed by an aggregation assay of blood cells, it is a time-consuming process that requires some skill to judge a positive result. If the cell array method is realized, washing and staining processes can be automated and the assay system will be conveniently performed. Recently, an automatic analysis using an allergen-microarray was reported [21].

2. Materials and methods

2.1. Preparation of photoreactive polymer

Photoreactive polymer was prepared as previously reported [33]. A copolymer consisting of 2-methacryloyloxyethyl phosphorylcholine (90 mol%) and methacrylic acid (10 mol%) was obtained from NOF Co. (Tokyo, Japan); it is referred to as PMAc. The molecular weight of PMAc, as measured by gel permeation chromatography, was 2.2×10^5 . Modification of PMAc was performed as follows. 4-Azidoaniline (12.44 mg) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (17.47 mg), purchased from Dojindo Lab. (Kumamoto, Japan), were dissolved in 2 mL of PMAc solution (5 wt%); 98 mL of water was added to the solution. The solution was left to stand for 24 h. After reaction, the product was dialyzed with a dialysis cassette (PIERCE, Rockford, IL, USA) until ultraviolet absorption confirmed no further release of azidoaniline through the cassette. The resultant solution was freeze-dried. The azidophenyl-derivatized PMAc is referred to as Az-PMAc. Elemental analysis indicated that the amount of azidophenyl group in Az-PMAc was 6%.

2.2. Preparation of cell array

Cell array was carried out as follows. Cells carrying M, N, A, B, AB, O, or Rh (D) antigen were chosen and nine kinds of panel cells, M+, N+, A; D+, B; D+, AB; D+, O; D+, O; D–, D+, and D– were employed. The symbols + or – mean the presence or absence of antigen on the cells, respectively. The cells were washed twice with phosphate buffered saline (PBS). After the washed cells were incubated in 2 vol% glutaraldehyde solution for 15 min or left untreated, they were mixed with 1 wt% of photoreactive polymer. The suspension (0.5 μ L) was spotted on a tissue culture polystyrene dish (diameter 35 mm, 3000-035X) purchased from Iwaki Glass Co., Ltd. (Chiba, Japan). After drying in air, the spot was immobilized by UV irradiation. The immobilized cell spot was washed three times with PBS containing 0.1 wt% Tween 20.

2.3. Binding assay of antibodies

Mouse antibodies or human sera were prepared as follows. In the case of anti-M, -A, -B, or -GPA, mouse (BALB/c) was immunized with each human erythrocyte and the immunized spleen cell was fused with a myeloma cell (NS-1). The fused cell was repeatedly cloned, each antibody-producing cell was established, and each antibody was collected from the cell. In the case of anti-D antibody, B lymphocyte isolated from humans carrying anti-D antibody was transformed with Epstein-Barr virus, and the transformed cell was cultured and fused with the myeloma cell. The fused cell was repeatedly cloned, an antibody-producing cell was established, and the antibody was collected. In the case of human sera, they were collected from healthy volunteers.

Binding assay was performed as follows. PBS containing anti-M, -A, -B, -D, or -glycophorin A (GPA) mouse monoclonal antibody (53, 62, 57, 58, and 86 mg/L, respectively), or human serum of A, B, O, or AB was added to the cell array plate and incubated at 22 °C for 2 h. After

incubation, the plate was washed with PBS containing 0.1 wt% Tween 20. Subsequently, the plate was incubated with a PBS containing anti- or -human whole antibodies IgG, linked with horseradish peroxidase (Amersham Bioscience Co., Piscataway, NJ, USA) at 22 °C for 1 h. After washing three times with PBS containing 0.1 wt% Tween 20, ECL reagents (Amersham Bioscience Co.) were added. The plate was analyzed using a chemiluminescence image analyzer, $n = 4$.

3. Results and discussion

First, the M+ cells were fixed with glutaraldehyde and mixed with PMAc or Az-PMAc, and the mixture was arrayed on a tissue culture polystyrene plate for the binding assay of monoclonal anti-M antibody. It was found that anti-M antibody bound to the spot of M+ cells immobilized with Az-PMAc, but not with PMAc. Because the cells were immobilized by the photocrosslinking reaction, the cells were not considered to be washed out during the binding assay. This result indicates that photoimmobilization was important for the binding assay.

Second, the binding of anti-M and -GPA antibody to the glutaraldehyde-fixed and photoimmobilized M+ or N+ cells was investigated. Anti-GPA was used as a control and it recognized both immobilized M+ and N+ cells. On the other hand, anti-M antibody bound to immobilized M+ cells, but not to N+. These results demonstrate that the recognition of antibody with the cells was specific. Dilution of the cells for the immobilization reduced the amount of bound antibodies.

Third, the effect of glutaraldehyde treatment on antigens locating on panel cells was investigated. Fig. 1 shows that anti-A and -B antibodies bound to A and B cells, respectively. Anti-D antibody bound to D+-type cells, but not to D--type cells. In this case, because all the A, B, and AB cells have D antigen, anti-D antibody bound to all the D+ panel cells. However, a comparison of Figs. 1a and b shows that the amount of anti-D antibody bound to cells treated with glutaraldehyde (Fig. 1a) was lower than for those that were not treated (Fig. 1b). D antigen is a membrane protein with a complex structure and anti-D antibody recognizes the three-dimensional structure of D antigen. For recognition of D antigen, glutaraldehyde treatment was not appropriate. Considering that glutaraldehyde fixation affects the molecular recognition, the fixation was not carried out before photoimmobilization for further experiments.

Fig. 2 shows photographs indicating monoclonal antibodies bound to spotted panel cells that were photoimmobilized with Az-PMAc. Anti-A and -B antibodies bound to the immobilized A; D+ and B; D+ cells, respectively. In addition, the antibodies bound to the AB cells. On the other hand, in this case, because O; D– cells had no D antigen, no reaction of anti-D antibody was found. These phenomena correspond to the result of an aggregation test for conventional blood types.

Finally, the array assay was used for human serum, as shown in Fig. 3. O-type serum reacted with A, B, and AB type cells. A- and B-type serum reacted with B and A cells,

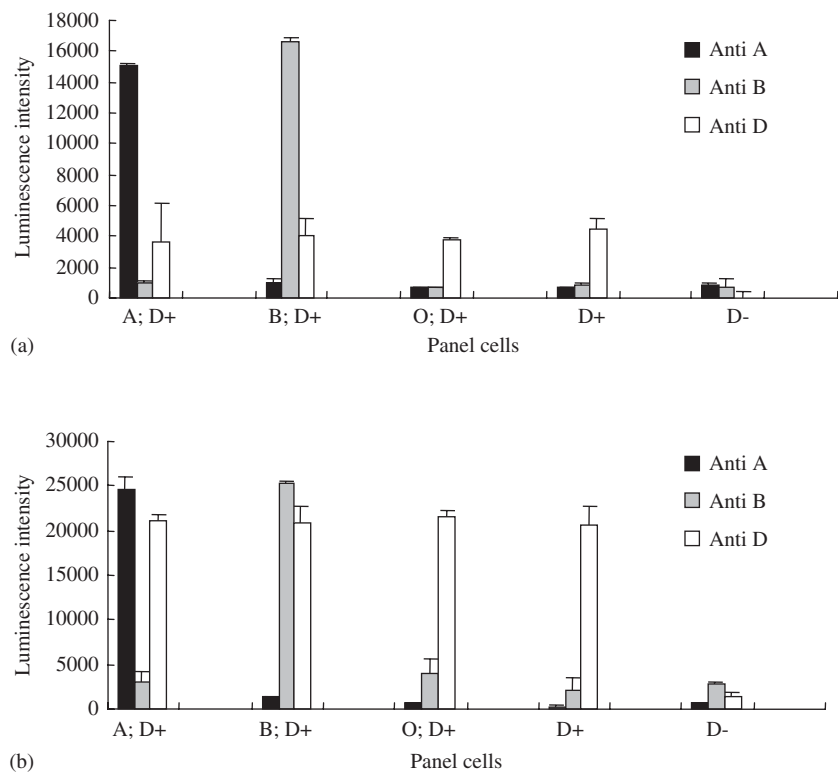


Fig. 1. Effect of glutaraldehyde fixation of panel cells on binding of antibody. (a) Glutaraldehyde-fixed panel cells were arrayed and (b) nonfixed panel cells were arrayed. Binding of anti-A (black), anti-B (gray), and anti-D (white) antibodies onto the cells.

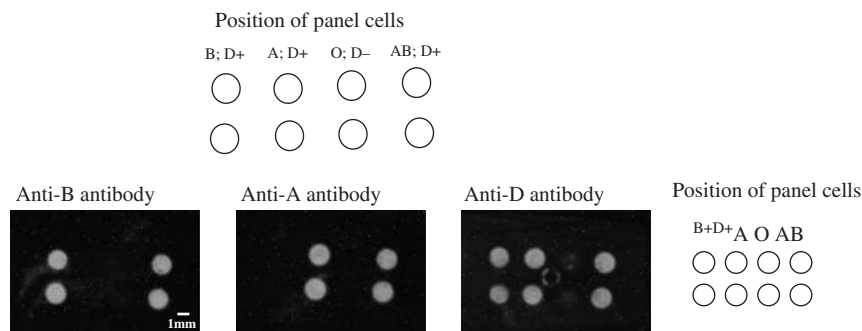


Fig. 2. Detection of chemiluminescence of antibodies bound on photoimmobilized panel cells array.

respectively, about 2-fold as high as with other cells. This is because A- and B-type serum have anti-B and -A antibody, respectively. AB-type serum did not react with either type of cell, because it has no antibody. Because O-type serum has both anti-A and -B antibodies, the panel cells except for O; D+ cells were stained. These phenomena correspond to the result of an aggregation test for conventional blood types.

Recently, Zhang et al. reported the capture of red blood cells by blood group antibodies (anti-A and -B) [34]. Conversely, this study has demonstrated that cells can be arrayed and immobilized on a plate and that the immobilized array was successfully employed for antibody detection, although some treatment of cells, for example

glutaraldehyde fixation, depends on the characteristics of the antigen. The photoimmobilization method will be useful in the array technique as an “everything array method”, even for cells.

4. Conclusions

A panel cell-microarray was prepared by using a photoimmobilization method. A photoreactive polymer was mixed with different types of red blood cells and the mixture was microspotted on substrate and photoirradiated. By this method the cells were immobilized on the substrate. Human serum was added to the cell-arrayed plate and adsorbed antibodies in the serum were detected

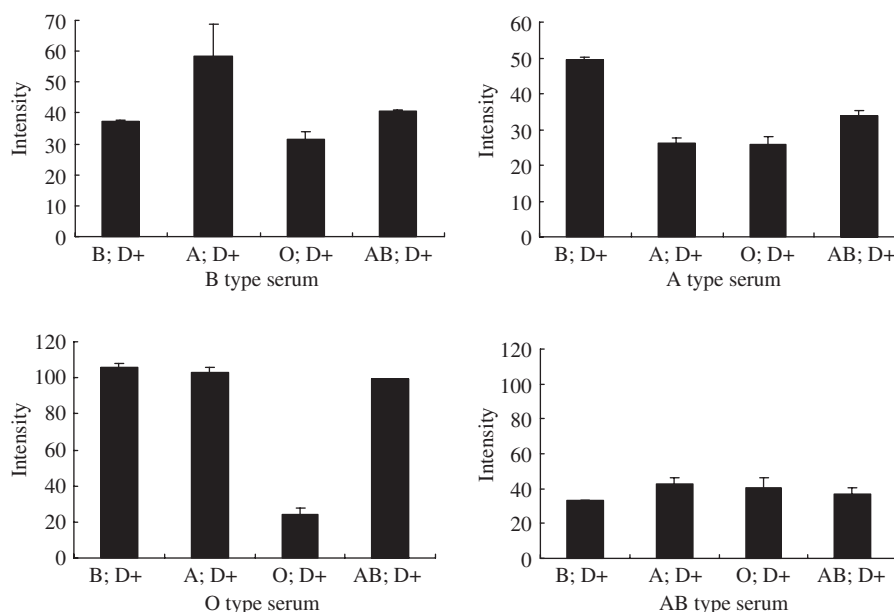


Fig. 3. Detection of chemiluminescence of antibodies in the serum of some blood types bound on photoimmobilized panel cells array.

as expected. The method using a cell array is useful for antibody detection because of its convenient multiple analysis of antibodies in human serum.

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