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Microarray preparation based on oxidation of agarose-gel and subsequent enzyme immunoassay

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Abstract

Based on molecular assembly technique, a thin film with double functions was prepared by using agarose gel as matrix on surfaces of glass slides. This process involved drying, then oxidation with $NaIO_4$ and subsequent coupling with glutaraldehyde. The aldehyde groups, derived from the film of the gel–matrix, could covalently link with the amino-groups in the molecule of various antigens (e.g. recombinant peptide, phospholipid, DNA), horseradish peroxydase and antibodies through formation of *Schiff's bases*. AFM characterization and assay results demonstrated that the gel assembly film was a surface of three-dimensional structure, and had a higher capacity for protein loading and linking than that of two-dimensional surfaces. In the light of the above principles, a biomolecular microarray was generated on this surface. By optimizing 100 mg/l was chosen as the concentration and 50 nl as the volume for spotting samples of diversified biomolecules. The enzyme–immunoassay system on the surface of gel–matrix by which the interactions of antigen–antibody and enzyme–substrate molecules were detected sensitively and specifically, was successfully developed by various experimental set-ups. The two main distinct advantages of the microchip are: (a) saving on volume of specimen and reagent, and (b) simultaneous analysis for multi-autoantibodies. Since the enzyme–substrate (HRP–DAB–H_2O_2) was used as the display system of signals from the microchip, the results were directly visible and more stable in comparison with fluorescent immunoassay.

Keywords: Microarrays; Antigen; Antibody; Enzyme immunoassay; Agarose gel

1. Introduction

The slide-supported biochip is a microarray format of the assembly of various biological compounds. Due to progress, such as microelectronic processing and molecular assembly, the field of microarrays has attracted much interest [1]. This novel and versatile technique allows high-throughput detection for DNA and proteins. The chip for immunoassay of protein is different from that of DNA, the principle of this assay is based on interaction of antigen–antibody or partner–receptor; the target molecules detected are only structure-specific but not sequence-specific [2,3]. At present, there is a variety of different methods for the detection of antigens and antibodies, e.g. immunogold filtration assay (IGFA), enzyme immunoassay (EIA), radio immunoassay (RIA), etc. All these methods are suitable for the detection of single items only, i.e. only a single antigen or antibody can be detected during each determination. The sensitivity of IGFA is lower, RIA has the disadvantage of generating radioactive waste, and is unstable. Overall performance of both methods is unsatisfactory. EIA technique has been widely used in laboratories, but is susceptible to interference by various factors. Thus, the development of a sensitive, stable and high-throughput method for antigens or antibodies was essential.

For the generation of high density protein arrays, Lueking et al. developed a technique for high-throughput gene expression and antibody screening on chip-size protein microarrays; they utilized robotic technology to array bacterical lysates of 92 human foetal brain cDNA (hEx1) which were subsequently blotted onto PVDF. Specific purified pro-

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tein wasgenerate protein microarrays could achieve high-throughput analysis for the antigen–antibody and partner–receptor interaction. However, for some smaller sized haptens, such as phospholipids, recombinant peptides, DNA and other proteins, which are immobilizing onto the solid phase simultaneously, there are no research results published yet; thus many problems remain to be solved in future work.

We prefer the agarose gel matrix and molecular assembly technique to generate a self-assembly membrane on glass slides by physical and chemical modification. The active groups at the surface could link with a variety of biological compounds. By related processing techniques, the antigens or antibodies, which immobilized on the solid support retain their original conformations and functional stability. In accordance with the above principles, we prepared microarrays, in order to obtain a high-throughput analysis for antigens or antibodies.

2. Experimental procedure

2.1. Samples, antigens and experimental materials

The pooled human serum which included a variety of autoantibodies was produced from clinical screening with ELISA; polyclonal rabbit anti-calmodulin serum was obtained by immunization with recombinant human calmodulin (rhCaM). Both of them were used as positive control. Normal rabbit serum served for negative control. All samples were prepared as stock and were kept at −20 °C.

Recombinant human calmodulin (rhCaM), recombinant human cyclophilin (rhCyP), human IgG, human thyroglobulin (TG), human thyroid peroxidase (TPO), human liver specific lipoprotein (LSP), human endometrial antigen (EmAg), human spermatozoa antigen (SmAg), human ovarian antigen (O-Ag), human myoglobin (Mb), rabbit IgG, cardiolipin, extractable nuclear antigen (ENA, obtained from fetal calf thymus), heat aggregation rabbit IgG (HAgRIgG) and rabbit anti-human IgG immunoglobulin: all of the above compounds were prepared by ourselves. Double stranded DNA (dsDNA) of fetal calf thymus was purchased from S_{ABC}, single stranded DNA (ssDNA) was prepared by thermal denaturation. Glutaraldehyde (GA), poly-L-lysine (PLL), bovine serum albumin (BSA), histone, agarose in which a variety of compounds were immobilized, and horseradish peroxidase (HRP)–conjugated polyclonal antibodies (anti-human IgG and anti-rabbit IgG) were purchased from companies as shown in Table 1.

2.2. Agarose-gel layer and glass slides preparation

The clean glass slides were immersed into 2% γ-aminopropyl-triethoxy silane (APTES) in acetone for 2 min at room temperature, then washed in acetone three times for 5 min each, and rinsed with ether [4]. After drying, they were covered with 1% agarose-gel containing 1% formaldehyde in 0.05 mol/l barbitone buffer (pH 8.6), 2 ml for each slide. Slides were frozen, then oven dried at 37 °C, followed by immersion into 0.1 mol/l $NaIO_4$ water solution for 20 min at room temperature, then washed in water three times for 15 min each, and again immersed in 1% glutaradehyde in 0.01 mol/l PBS, pH 7.4 for 4 h at 37 °C (APTES–GA and PLL–GA modified slides were prepared as the same step by glutaradehyde); finally, slides were washed for preparation as before.

2.3. AFM characterization and contact-angle measurement

Atomic force microscopy (AFM) was used to obtain three-dimensional surface profiles of the microarray on slide and a commercial system (Nanoscope IIIa, Digital Instruments, Santa Barbara) was operated in the contact mode [5]. A 16 μm scanner was used for surface inspection. The soft cantilevers were 200 μm length with an integrated pyramidal Si_3N_4 tip and a spring constant of 0.12 N/m. Typical forces for all measurements were of the order of approximately 1 nN or less. Surface contact-angle measurement was performed by using Rane-Hart 100–00230 Instrument.

2.4. Biomoleculars immobilization on solid support

For preparing microarrays, we used a 2 μl pipette to deliver 50 nanoliter volumes of different concentration samples to different surface of solid supports (including agarose-gel surface, APTES–GA, APTES, PLL–GA and PLL modified slides), yielding spots about 0.5–1.0 mm in diameter. All of the antigens and antibodies were prepared in 0.05 mol/l, pH 9.6 carbonate-buffered saline with 40% glycerol. After 4 h of incubation at 37 °C, the slides were washed with water,

Table 1
Array antigens, corresponding antibodies and experimental materials

Array antigens	Sources	Corresponding antibodies	Sources	Reagents	Sources
dsDNA	S_{ABC}	HRP-goat anti-human IgG	SHHBPI	Agarose	Sigma
ssDNA	S_{ABC}	HRP-goat anti-rabbit IgG	Sigma	APTES	Sigma
histone	Promega	Rabbit anti-human IgG	By ourselves	Glutaraldehyde	Sigma
BSA	Sigma	Rabbit anti-rhCaM serum	By ourselves	DAB	Sigma
HRP	Sigma	APTES/and PLL modified slides	Sigma		
PLL	Sigma	APTES–GA/and PLL–GA modified slides by ourselves			

S_{ABC}: Sino-American Biotechnology Company; SHHBPI: Shanghai Biological Products Institute.

PBS and water for 5 min each, respectively. The microarrays were blocked by incubation in PBS buffer containing 0.1% BSA, 1% glycine and 0.01 mol/l $NaBH_4$, for 2 h at 4 °C, then rinsed with PBS. These slides were stored at 4 °C.

2.5. Immunoassays on the microarrays

Immuno-and enzymatic assays on microarrays were usually carried out in a 200 μl chamber. Thus, usually 200 μl of solution was taken for each step of an assay. The main procedure of the immunoassay was performed as follows. The blocked microarrays were incubated with diluted samples (dilution factor: 10–100) for 30 min at 37 °C, the microchips were then washed three times for 5 min each with PBS buffer containing 0.05% Tween-20, and then incubated with HRP-conjugated secondary antibodies (GAHIgG or GARIgG) for 20 min at 37 °C, then washed as in the previous step. HRP activity on the microarrays was detected with 5 mmol/l 3,3′-diaminobenzidine tetrahydrochloride (DAB) and 0.03% H_2O_2 in 50 mmol/l PBS buffer, pH 7.4, for 5–10 min. The positive reactions were detected as black spots. Images of microarray were acquired with F/B scanner (Mustek, V3.7 Taiwan). The analysis for microarray spots was performed with Adobe Photoshop to recognize and quantify the spots. Intensities of spots were transferred to Excel spreadsheets in order to display results in form of curve–diagrams.

2.6. ELISA for autoantibodies detection

Fifteen of the above antigens were coated on polystyrene plates and their corresponding autoantibodies in pooled serum were detected by ELISA method as described in literature [6].

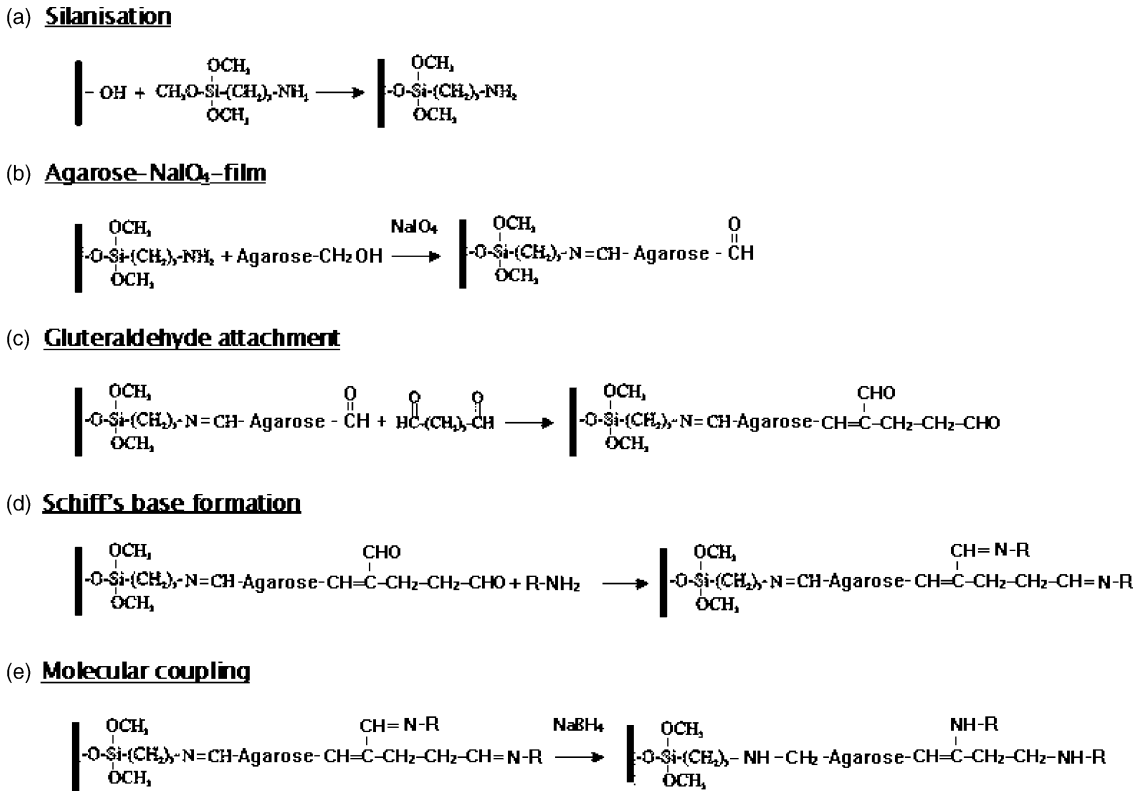
3. Results

3.1. The principle of agarose linked with biomolecules

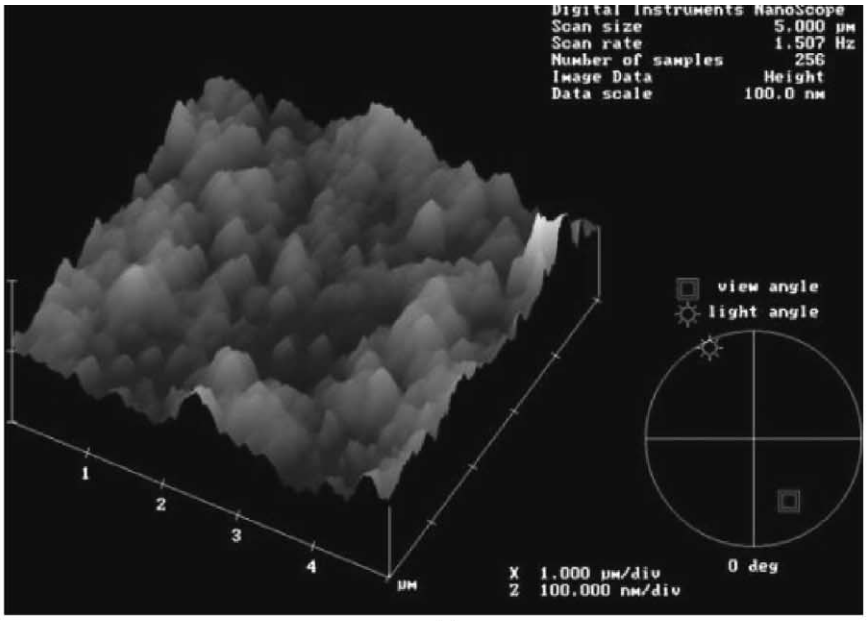
Agarose gel deposited on glass slides to form a thin layer after oxidation by $NaIO_4$ and linked with GA. The gel matrix provided molecular layer with double functions. The derived active groups on the surface of gel linked with various biological compounds, such as peptide, phospholipid, DNA, protein and antibody. The scheme of molecular assembly on glass slides are as following (see Scheme 1).

3.2. AFM characterization and contact-angle measurement

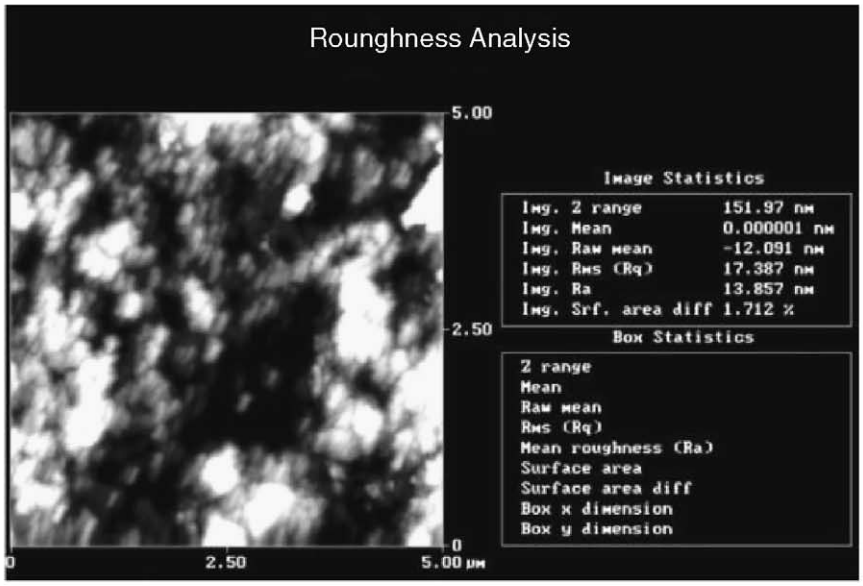
The surface profiles of the microarray characterized by AFM showed that oxidized agarose gel film was a kind of three-dimensional solid support (Fig. 1a). The mean roughness of gel surface was 13.857 nm. The section analysis



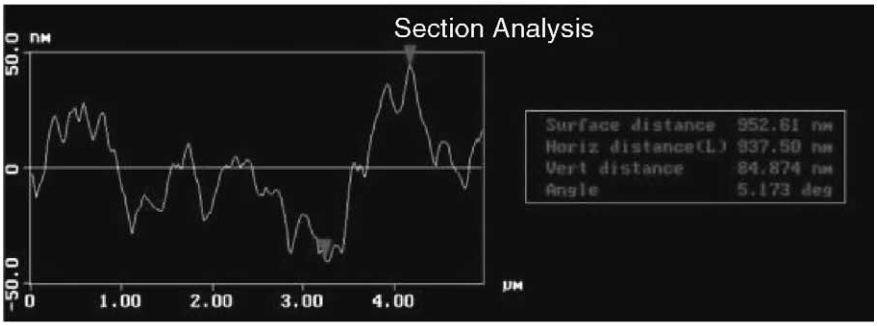
Scheme 1. Schematic protocol for immobilization of biomoleculars onto modified surface. This protocol include following steps: (a) glass slides silanisation, (b) agarose-gel layer preparation and oxidated by $NaIO_4$, (c) gluteraldehyde attachment with oxidated agarose, (d) *Schiff's base* formation between aldehyde- and free amino-groups and (e) molecular coupling and immobilized on agarose-gel surface.



(a)



(b)



(c)

Fig. 1. Agarose-gel membrane AFM image (5 μm × 5 μm): (a) three-dimensional image, (b) roughness analysis for agarose-gel layer (two-dimensional image) and (c) section analysis for agarose-gel layer.

for agarosegel layer revealed that its vertical distance was 84.874 nm (Fig. 1b and c). So, it was considered that the thickness of film surface was roughly estimated. The surface contact-angle was about $26 \pm 2^{\circ}$ in agarose gel, while it was 34° and 12° in both APTES and PLL modified and both APTES–GA and PLL–GA modified slides, respectively.

3.3. Horseradish peroxidase microarray

The horseradish peroxidase being widely used in immunoassay, was tested for immobilization on microarrays by five different solid supports. The activity of solid phase enzyme was detected with specific substrate yielding colored precipitate during enzymatic reactions. HRP was immobilized for 4 h at 37 °C, at a concentration of 0.1–1000 mg/l and delivered as 50 nl sample for each spot. The microarrays were covered with a solution of 3,3′-diaminobenzidine tetrahydrochloride–H_2O_2, and the signal of the insoluble brown precipitate was observed as proper spot. The results showed that only agarose-gel surface could provide with the highest sensitivity of interaction between horseradish peroxidase and diaminobenzidine tetrahydrochloride–H_2O, when immobilized HRP concentration is lower to 5 mg/l, the specific signals could also be observed (Fig. 2). So, in the process of subsequent experiment, we selected the agarose-gel surface and used it as a solid support for microarray preparation.

3.4. Antigen detection by direct enzyme immunoassay

The antigen of human IgG was immobilized on the surface of the modified matrix at different concentrations (1–5000 mg/l). At constant secondary antibody concentration labeled with HRP, we optimized the spotting concentrations of antigen at 100 mg/l, and sample spotting volume at 50 nl (Fig. 3a). Thus, in the following antigens'

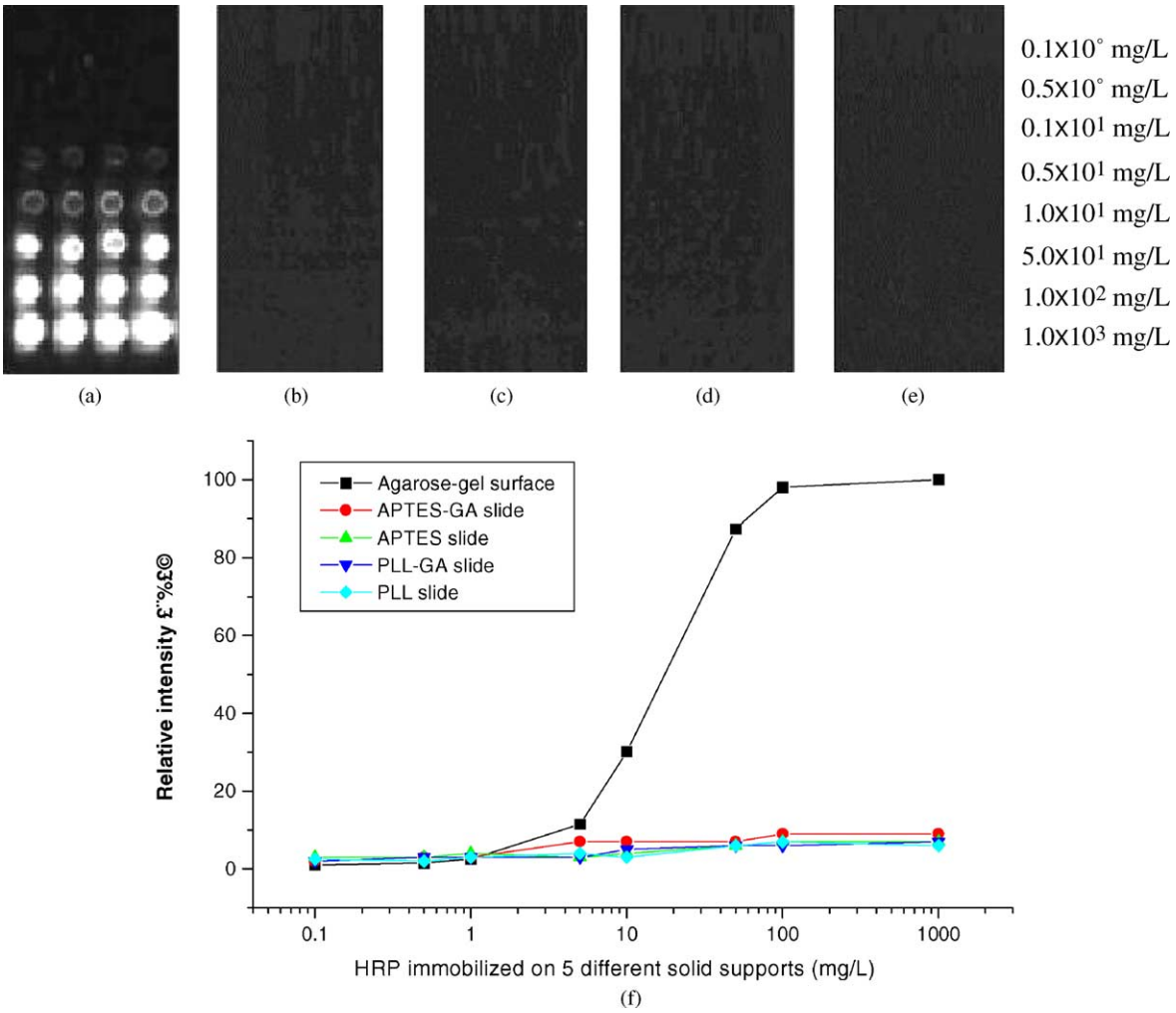


Fig. 2. Horseradish peroxidase microarray. The interaction between horseradish peroxidase and diaminobenzidine tetrahydrochloride–H_2O_2, horseradish peroxidase was immobilized at a concentration of 0.1–1000 mg/l and delivered as 50 nl sample for each spot. (a) $NaIO_4$ oxidized agarose-gel and subsequently modified by GA slide, (b) APTES–GA modified slide, (c) APTES modified slide, (d) PLL–GA modified slide, (e) PLL modified slide and (f) the effect of horseradish peroxidase concentration used for the immobilization on its activity: the average relative intensity of spots of horseradish peroxidase correlated nearly linearly with the concentrations of immobilized enzyme (only agarose-gel surface).

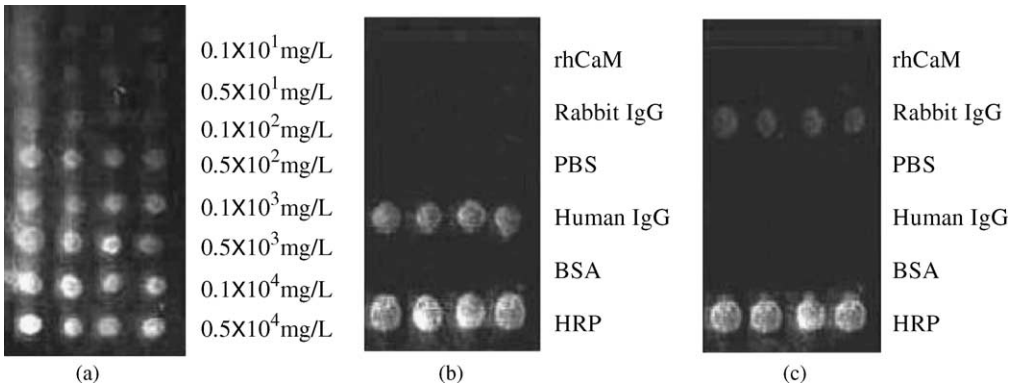


Fig. 3. Antigen detection by direct enzyme immunoassay: (a) human IgG was immobilized on the microarray at a concentration of 1–5000 mg/l and was detected with goat anti-human IgG-HRP, (b) different antigens containing rhCaM, rabbit IgG, human IgG and BSA were immobilized, and detected with goat anti-human IgG-HRP, horseradish peroxidase was immobilized as a control and (c) different antigens containing rhCaM, rabbit IgG, human IgG and BSA were immobilized, and detected with goat anti-rabbit IgG-HRP, horseradish peroxidase was immobilized as a control.

or antibodies' microarrays, we usually utilized this concentration and volume, and antigens were detected by two different secondary antibodies (goat anti-human IgG and goat anti-rabbit IgG), which were labeled with HRP, respectively. Results showed that the secondary antibodies could bind to the corresponding antigens (human IgG and rabbit IgG) that were immobilized on solid support, and no interactions with other antigens were observed in the microarray (Fig. 3b and c).

3.5. Free antibody detection by indirect enzyme immunoassay

Polyclonal rabbit anti-CaM serum was tested with this microchip which reacted with rhCaM, human IgG, BSA and HRP, also responding to positive control. Only specific interaction was observed (Fig. 4a). Thus, due to absence of anti-CaM-antibody in normal rabbit serum, the rhCaM spots in the microarray gave no colored reactions (Fig. 4b).

To investigate the specificity of this assay, we also tested the pooled serum containing a variety of human autoantibodies. The corresponding autoantibodies showed brown positive spots. Furthermore, the alien animal compound antibody against rabbit IgG was observed in the human serum. Fig. 5a and b shows the detection of autoantibodies in serum of different dilutions, the antibodies to thyroglobulin and calmodulin reacted positively. The antibody to rabbit IgG gave negative results when the test serum was diluted 1:100.

Further support for the specificity of this microarray comes from the following findings: preincubation of test serum with normal rabbit IgG with a final concentration of 200 mg/l inhibited corresponding antibody binding to the rabbit IgG on the solid phase by 100%, and its original positive spots completely disappeared (Fig. 5c).

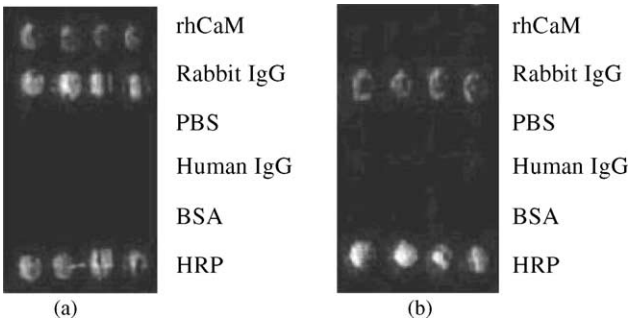


Fig. 4. CaM-antibody detection by indirect enzyme immunoassay. Microarrays with immobilized rhCaM, rabbit IgG and human IgG covered with rabbit anti-rhCaM serum (a) or normal rabbit serum (b), and subsequently hybridized with goat anti-rabbit IgG-HRP; horseradish peroxidase and BSA were immobilized as controls and test sera were diluted 1:50.

3.6. Free antigen detection by sandwich immunoassay

Polyclonal rabbit anti-human IgG immunoglobulin was immobilized on solid support to prepare various microarrays. The microarrays were covered with solutions of human IgG at concentrations of 0.01–10,000 mg/l. After washing, the microarrays were incubated with HRP-conjugated goat anti-human IgG. Specific interaction could only be observed in microarrays, which were covered with the human IgG solution. The intensity of spot signals increased upon increasing the human IgG concentrations, and was positive correlated with IgG concentrations significantly (Fig. 6a). Intensities of spots were transferred to Excel spreadsheets in order to display results in form of curve–diagrams (Fig. 6b). Its regression equation was $Y = 5.731 \ln x + 45.777$, and its relative coefficient was 0.997, the lowest limit which detected by this microarray was 0.01 mg/l.

3.7. Multi-autoantibody detection by enzyme immunoassay

The microarray was designed for 96 lattice (8 × 12 pattern), and 17 different compounds were immobilized on the surface of modified agarose-gel. Each antigen was applied in a 2 × 2 spotting pattern, yielding spots about 0.5 mm

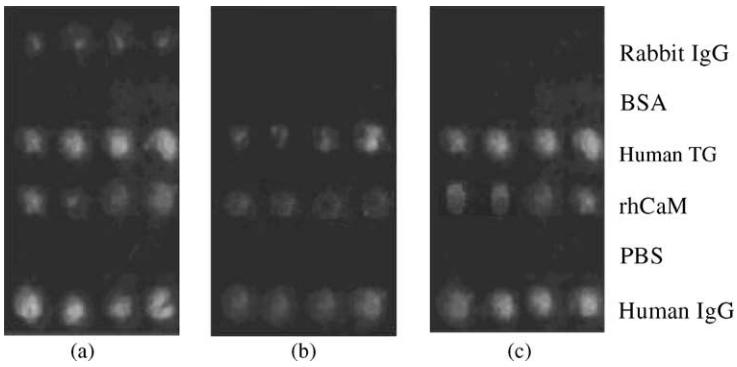


Fig. 5. Human autoantibody detection by indirect enzyme immunoassay. The microarrays with immobilized rabbit IgG, human TG and rhCaM, and covered with pooled serum containing a variety of human autoantibodies, and subsequently developed with goat anti-human IgG-HRP; human IgG and BSA were immobilized as a control. (a) Test serum dilution 1:10, (b) test serum dilution 1:100 and (c) inhibition test with rabbit IgG and test serum dilution 1:10.

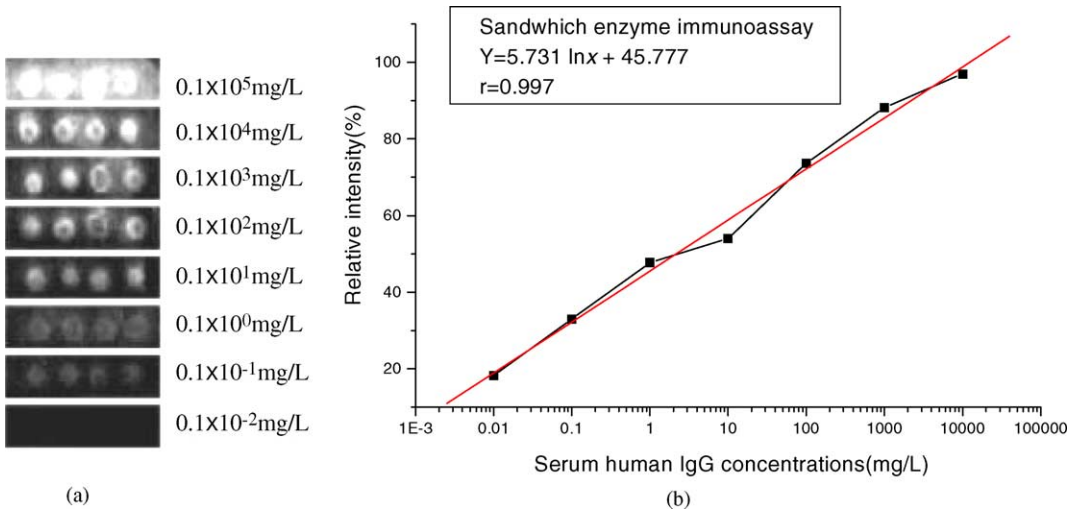


Fig. 6. Human IgG detection by sandwich immunoassay: (a) polyclonal rabbit anti-human IgG immunoglobulin was immobilized, and incubated with human IgG solutions with concentrations of 0.01–10,000 mg/l, and detected with HRP-conjugated goat anti-human IgG, (b) the average intensity of specific signals which detected by using microarrays were positive correlated with IgG concentrations significantly, its regression equation was $Y = 5.731 \ln x + 45.777$, and its correlation coefficient was 0.997.

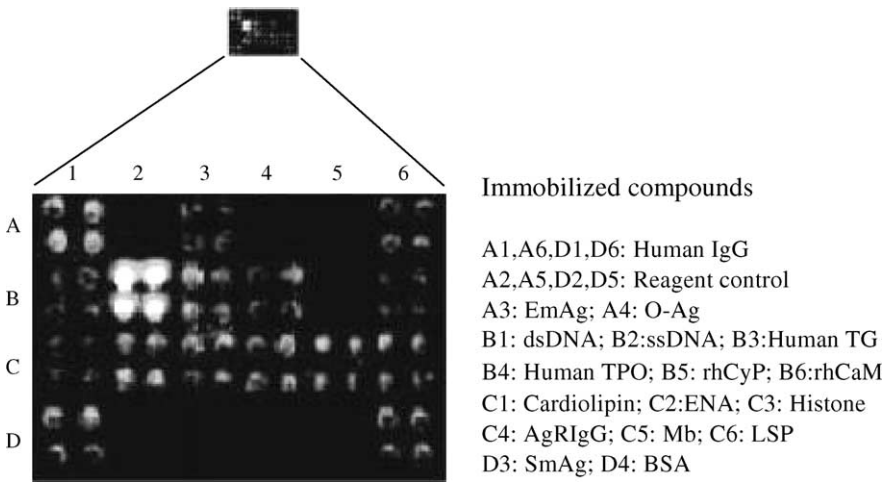


Fig. 7. Multi-autoantibodies detection on one microarray. The microarray was designed for 96 lattice (8 × 12 pattern), and immobilized 17 different biological compounds, subsequently incubated with pooled serum and goat anti-human IgG-HRP, human IgG and BSA were immobilized as a control, the test serum was diluted 1:50.

Table 2
Comparison of microarray and ELISA assay for autoantibodies

Sample	Autoantibodies against corresponding antigens (array detection/ELISA assay)														
	EmAg	AoAg	AsAg	dsDNA	ssDNA	TG	TPO	rhCyP	rhCaM	Cardiolipin	ENA	Histone	AgRIgG	Mb	LSP
1	−/−	−/−	−/−	+/+	+/±	−/−	+/±	−/−	+/±	−/−	+/±	+/+	+/+	−/−	+/+
2	±/±	−/−	−/−	+/+	+/+	+/+	+/±	−/−	+/+	+/+	+/±	+/+	+/+	+/+	+/+

in diameter, and spot-to-spot distance was 0.5 mm. Human IgG located on the four corners of microarrays served as a positive control, whereas BSA spots represented negative control, and compounds of known autoantigens were scattered in the middle of the microarray. The microarray-based enzyme immunoassay was carried out as described in materials and methods. Fig. 7 shows the result of autoantibody signals in pooled serum, only the antibodies against ovarian, cyclophilin and spermatozoa reacted negatively, the other autoantibodies gave all positive signals.

3.8. Comparison of microarray and ELISA in assay for autoantibodies

To assess availability of the microarray in immunological assay, two pooled sera containing a variety of autoantibodies were tested by microarray and ELISA simultaneously. The results revealed that in sample 1 the following autoantibodies: anti-ssDNA, -ssDNA, -TPO, -rhCaM, -ENA, -histone, -AgRIgG and -LSP were detected by both the two methods. In sample 2 all the autoantibodies were positive with the exception of antibodies to ovarian, cyclophilin and spermatozoa. Furthermore, the detectable positive autoantibodies in the 2 samples by the two methods were primarily consistent (see Table 2). So, it was considered that the microarray based on this oxidized agarose-gel film was suitable for the detection of antigen–antibody interactions.

4. Discussion

There are many kinds of methods for immobilization of protein or antigen, such as the physical and chemical procedures [7]. The former is mainly based on passive adsorptionin which the adsorbed protein is not uniform and is more easily to be taken off from the solid surface. So, it is usually rejected for protein or antigen immobilization. At present, the chemical methods are usually used for the immobilization of bioactive organic molecules. Its principle is mainly based on cross-linking by covalent linkage and then fixed onto the solid surface, normally compounds that immobilized can provide with uniform space tropism and distribution. Because of the matrix presenting specific physical and chemical features, agarose-gel has been widely used in the field of life science and its associated research, and is the ideal material for immobilization as well as electrophoresis. Agarose was alternately formed of 1,3-β-D-galactopyranose and 1,4-3,6-dehydration-α-D-galactopyranose. When the gel is condensing, no polymerization of free radicals occurred; consequently, no catalytic promoters are needed [8]. The results of this study showed that only agarose-gel surface could provide with the highest sensitivity of interaction between horseradish peroxidase and diaminobenzidine tetrahydrochloride–H_2O. When immobilized HRP concentration was lower to 5 mg/l, the specific signals could also be observed (Fig. 1). So, we think that agarose support, i.e. oxidized agarose-gel surface and subsequently modified by glutaraldehyde, was superior to other 4 kinds of slides for protein immobilization, and it will also be a novel application for agarose-gel.

The active groups derived from gel-surface could link with biological compounds. When samples buffer containing protein/or other antigens were spotted onto this surface, the spotted area then gradually changed into semisolid estate. The aldehyde groups derived from the support could covalently link with the free amino-groups in the molecule of proteins or antigens. On the other hand, compounds could also infiltrate into micro-pores of agarose-gel. So, this gel–matrix has a higher capacity for protein/or antigen loading and linking than that of two-dimensional surfaces (such as PLL–GA or APTES–GA modified slides). Owing to this gel–matrix providing with quality of relative hydrophile, the immobilized proteins or antigens are situated in water–gelatinous environment and well spaced. This similar solution circumstance could prevent aggregation of immobilized compounds or their interphase-induced denaturation and is highly advantaged to subsequent interactions of antigen–antibody [9]. Moreover, the matrix is also suitable for the effective fixation of other different molecules, such as recombinant peptides, phospholipids and DNA, etc. After oxidation and modification, its anti-splitting intensity against water and chemical reagents as well as viscosity properties was significantly improved. Although the production of microarray was performed by incubation, immersion in solutions and washing for several times, the agarose-gel on the glass slides do not split or take off, and is suitable for scanning with optical devices as well as for long-term storage.

In addition to surface-active groups that could be used, biological compounds immobilization was highly related to other physical and chemical characteristics, e.g. the qualities of surface hydrophile and hydrophobe, whereas the gel thickness was not main influence factor for their cross-linking. AFM characterization and assay results showed that oxidized agarose-gel film was a kind of three-dimensional

surface, its mean roughness was 13.857 nm, and contact-angle was 26°. Section analysis revealed that its vertical distance was 84.874 nm, this measurement could probably estimate the film surface status. In comparison with other four kinds of slides, the hydrophile of gel surface was better than those of APTES or PLL modified slides (both of their contact-angle was 34°). Whereas, its hydrophobe was better than those of APTES–GA or PLL–GA modified slides (both of their contact-angle was 12°). This characteristic was advantaged to proteins and solid support combination, and meanwhile no dispersing of array spots occurred in microarray.

In the process of microarray preparation, we utilized the carbonate-buffered saline with 40% glycerol to dissolve antigens and antibodies, the main reason for this consideration was as following. In the first place, it could prevent evaporation of the nanodroplets in microarrays, and was important for compounds to remain hydrated throughout this and subsequent steps to prevent denaturation and to retain conformations as well as functional stability [10]. Secondly, in the weak basic solutions, the aldehydes at the surface of the agarose-gel film could easily react with primary amines of the biological compounds to form *Schiff's base* linkages.

In this study, we developed several patterns for immunoassays on microarrays, and their application was performed for detecting antigens and antibodies. The main advantage of this technique was that autoantibody screening could be carried out simultaneously, in high-throughput, and highly economical in the use of specimens and reagents. In our work, the autoantibody detection was firstly introduced into a system based on microarray test, the enzyme and its specific substrate served as a detection system, and provided more stable and visible results than that of fluorescence. The autoantibodies in pooled serum were detected by this method, and compared with that of ELISA screening, both of them were primarily consistent (Table 2). In addition, low titer antibody against rabbit IgG was also observed in human serum. After the test serum was diluted or inhibited by corresponding antigen, the color spots that developed by binding of antibody to rabbit IgG disappeared. This technique is a sensitive and specific method which can well perform qualitative and quantitative sample analysis.

5. Conclusions

In conclusion, we can say, two prominent advantages of this microarray which used for detertion are: (a) saving in volume of specimen and reagent, and (b) simultaneous analysis for multi-autoantibodies. So, it is feasible to prepare microarrays on the surface of oxidated agarose-gel, this technique is suitable for multi-compounds analysis, especially autoantibody parallel detection, in high-throughput, and allows practical as well as wide spread applications.

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References

[1] G.H.W. Sanders, A. Manz, Chip-based microsystems for genomic and proteomic analysis, Trends Anal. Chem. 19 (6) (2000) 364–378.
[2] P. Arenkov, A. Kukhtin, A. Gemmell, S. Voloshchuk, Protein microchips: use for immunoassay and enzymatic reactions, Anal. Biochem. 278 (2000) 123–131.
[3] H. Ge, UPA, A universal protein array system for quantitative detection of protein–protein, protein–DNA, protein–RNA and protein–ligand interactions, Nucleic Acids Res. 28 (2000).
[4] M. Nisnevitch, M. Kolog-Gulco, D. Trombka, B.S. Green, M.A. Firer, Immobilization of antibodies onto glass wool, J. Chromatogr. B 738 (2000) 217–223.
[5] W. Qian, D. Yao, B. Xu, F. Yu, Z. Lu, W. Knoll, Atomic force microscopic studies of site-directed immunobilization of antibodies using their carbohydrate residues, Chem. Mater. 11 (6) (1999) 1399–1401.
[6] G. Shen, R. Zhou, Modern Immunology Experimental Technique, the front ed., Hubei Science Technology Press, Wuhan, 1998, pp. 210–224.
[7] B. Xu, W. Qian, Y. Lin, Z. Lu, Comparison of two different ELISA adsorbed methods on AFM and activity analysis, J. Clin. Lab. Sci. 17 (1) (1999) 83–85.
[8] Agarose Monograph, fourth ed., FMC Ltd., Rockland, USA, 1988, pp. 51–56.
[9] D. Guschin, G. Yershov, A. Zaslavsky, A. Gemmell, V. Shick, D. Prudnikov, P. Arenkov, A. Mirzabekov, Manual manufacturing of oligonucleotide microchips to enhance sequencing by hybridization, Anal. Biochem. 250 (1997) 203–211.
[10] G. MacBeath, S.L. Schreiber, Printing proteins as microarrays for high-throughput function determination, Science 289 (8) (2000) 1760–1763.