JAIST Repository
https://dspace.jaist.ac.jp/

Title	Probe-on-carriers for oligonucleotide microarrays (DNA chips)
Author(s)	Tsukahara, Toshifumi; Nagasawa, Hiroshi
Citation	Science and Technology of Advanced Materials, 5(3): 359-362
Issue Date	2004-05
Type	Journal Article
Text version	author
URL	http://hdl.handle.net/10119/4012
Rights	Elsevier Ltd, Toshifumi Tsukahara, Hiroshi Nagasawa, Science and Technology of Advanced Materials, 5(3), 2004, 359-362. http://www.science-direct.com/science/journal/14686996
Description	

JAIST
JAPAN
ADVANCED INSTITUTE OF
SCIENCE AND TECHNOLOGY

Japan Advanced Institute of Science and Technology

Probe-on-carriers for Oligonucleotide microarrays (DNA Chips)

Toshifumi Tsukahara[1,2,*] and Hiroshi Nagasawa[3]

[1]Center for Nano Materials and Technology, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Tatsunokuchi, Ishikawa 923-1292, Japan

[2]CREST, Japan Science and Technology Agency, Kawaguchi, Saitama 332-0012. Japan

[3] Precision Machinery Group, Ebara Corporation, 4-2-1 Honfujisawa, Fujisawa, Kanagawa 251-8502, Japan

Key words: oligonucleotide microarray; DNA Chip; oligonucleotide synthesis; hybridization; porous glass; probe-on-carrier; single nucleotide polymorphisms; SNPs

* Corresponding author. Tel. +81-761-51-1440; Fax. +81-761-51-1149

E-mail address: tukahara@jaist.ac.jp (T. Tsukahara).

Abstract

Oigonucleotide microarrays (DNA Chips) are very efficient tools to analyze genotypes of patients or change in gene expressions between two different samples. However, there is no cost effective procedure to manufacture DNA chips. We are developing “probe-on-carriers”, immobilized oligonucleotide probes on solid phase to make DNA chips. In this procedure, each oligonucleotide is synthesized on a controlled porous glass carrier as a solid phase, and can be used as a probe for each sequence. This can be substantiated by technology for strictly controlled pore-size of porous glass. In fact, we found the sequence specific hybridization of probe-on-carrier with using porous glass of larger than 50nm pore diameter.

The probe-on-carriers for wildtype and mutant *p53* genes were hybridized with their complementary probes, respectively, but not with another probes. This result clearly demonstrated that the probe-on-carriers could recognize one-nucleotide substitutions of a gene. We found that the fixed probe-on-carriers on a slide glass still showed sequence specific hybridization. Therefore, we conclude that the probe-on-carriers are epoch-making materials for making DNA chip economically.

1. Introduction

Oligonucleotide microarrays (DNA chips) are small devices containing gene fragments that are either synthesized directly onto or spotted onto glass or other substrates. Thousands of genes are usually represented in a single array. DNA chip-based hybridization analysis is a promising new technology to allow rapid and cost-effective analysis for genes. DNA chips are currently applied not only to analyze gene expression, clarify metabolic pathways and pathogenetic mechanisms but also to identify genotypes including single nucleotide polymorphisms (SNPs). SNP patterns associated with adverse drug reactions can be discovered. Therefore, it can be expected that pharmacogenetic research will identify situations where a drug should be avoided in certain individuals in order to reduce the risk for adverse drug reactions.

Oligo-DNA arrays are produced by using the semiconductor photolithography technology, patterned, light-directed combinatorial chemical synthesis on a small glass surface. A surface of a solid support modified with photo-labile protecting groups is illuminated through a photolithographic mask, yielding reactive hydroxyl groups in the illuminated region. An activated deoxynucleoside, protected at the 5’-hydroxyl with a

photo-labile group, is then presented to the surface and coupling occurs at sites that were exposed to light. The process is repeated until the entire number of oligonucleotides probes and control has been synthesized. However, this technology is very costly and time consuming, and the yield of each synthesized oligonucleotide was subject to wide variation and uncertainty. An alternate procedure is delivering and covalently attaching pre-synthesized oligonucleotides onto a surface of solid support. First, oligonucleotides are synthesized by standard method. Then chemical modification is introduced at 5'- or 3'-ends of each oligonucleotide. Finally, the oligonucleotides are covalently attached by hetero-bifunctional cross-linkers or by chemical coupling on a slid-glass for coupling. This technology is also costly because introduction of cross-linkers or functional residues to each pre-synthesize oligonucleotide by chemical modification are very expensive.

Usually, the oligonucleotide is synthesized on a support filled with porous glass or polystyrene or a membrane. Therefore, if we can use pre-synthesized oligonucleotide on a support it-self, without the cleavage as a probe for oligo-DNA array, we can manufacture oligo-DNA array easily and inexpensively. In this report, we

are developing “probe-on-carriers”, immobilized oligonucleotide probes on solid phase to make DNA chips. In this procedure, each oligonucleotide is synthesized on a solid phase, and can be used itself as a probe for each sequence.

2. Experimental

2.1. Materials

Several kind of porous glasses with a definite composition were prepared by heat and chemical treatment of borosilicate glass. The glass of the composition SiO_2; 62.5, B_2O_3: 27.3, $A1_20_3$: 3.0 and Na_20: 7.2 wt. % was melted, and were heat treated for phase separation at 600 ºC for various times (2h-7 day). The phase-separated specimens were leached in 0.5 N hydrochloric acid for 1 day, and were leached in 0.2 N sodium hydroxide for 1 hour. Pore distribution of porous glass used in the experiment are shown in Fig. 1. Particle size of porous glass was adjusted to 10 μm. This porous glass (10 g) was refluxed with glycidoxypropyltrimethoxysilane (1 wt.%) in toluene (50 ml) at 110 ºC, 10 hours with stirring, then washed thoroughly with methanol, ether and air-dried.

These derivatized porous glasses were heated with stirring in hexaethyleneglycol containing a catalytic amount of concentrated sulphuric acid, 16 hours in an atmosphere of nitrogen, at 80 ºC., to yield alkyl hydroxyl derivatized porous glass. After washing with methanol and ether, the porous glasses were dried under vacuum and stored under nitrogen at room temperature (4)(5).

A small amount of the hydroxyalkyl derivatized porous glass was put into the reaction vessel of an automatic oligonucleotide synthesizer (own composition), programmed to synthesize sequences. The first nucleotide became covalently attached to the derivatized porous glass. Measuring the amounts of trityl group removed, in the spectrophotometer can monitor each step in the synthesis. By this test, the stepwise yield was 90-95%. The product was deprotected by the standard treatment with hot ammonia and washed thoroughly with distilled water (6).

A polybutadien adhesive was spin-coated on a slid glass, then spot suspended solution of 3 kinds of probe-on-carriers to make oligo-DNA array by the fixation. The slide glass made dry to fix probe-on-carriers well, then washed by ultra-sonication to remove extra probe-on-carriers.

2.2. Hybridization

The sequence from 947th to 966th of human *p53* gene, 5'-GGCATGAACCG-GAGGCCCAT-3', was used as a target in this study. The sequences of mutants are followed. HSC-4: GGCATGAACCAGAGGCCCAT, and Ca9-22 mutant: GGCATG-AACTGGAGGCCCAT (7). Three kinds of probe-on-carriers were prepared by synthesis those sequences on pre-treated porous glass. On the other hand three kinds, native HSC-4 and Ca9-22, of fluorescent-labeled anti-sense oligo-DNAs were prepared by general protocol. Each FITC-labeled oligo-DNA was mixed with three kinds of pre-synthesized probe-on-carriers, and then hybridized each other at 55 ºC for over night. Then washed with 0.1X SSC at room temperature (8). Remaining fluorescence with each probe-on-carrier was observed by fluorescent microscope after stringency wash. Relative brightness was determined with using the Photoshop software, Adobe Systems Inc, as 0; black and 100; white.

3. Results and discussion

3.1 Probe-on-carriers

We think the best material for probe on-carrier is porous glass, because they are resistant to heat and chemical hazards, have low absorbency with peptides or nucleotides, and their pore-size could be controlled strictly. As shown in figure 1, pore-size of our porous glass could be controlled in a narrow range.

To synthesize oligonucleotides on porous glass, we used 3-glycidoxy-propyltrimethoxysilane coatings for probe-on-carriers and the hexaehylenglycol-linker for solid phase oligonucleotide synthesis in this study. The density of these silan-coupling reagents on glass surface was 1 micro mole/ m^2. To remove non-specific fluorescence, porous glass was preserved in methanol, THF, ammonia (1:1:1) for 2 days, then washed with triethylamine for 10 hrs.

There are most of reactive linkers should on surface of pores. Therefore, hybridization reaction should mainly occur within each pore. Because DNA molecules are huge, there must have enough space in each pore. In fact, we could not get good result with porous glass with 30 nm pore diameter (Fig. 2, the bottom row). We found

the sequence specific hybridization of probe-on-carrier with using porous glass of more than 50nm pore diameter. Therefore, porous glass with 50 or 100 nm in diameter was used in following studies.

We chose *p53* tumor suppressor gene as a target for experiments. Mutations on *p53* gene occur with unusually high frequency in tumor tissue. And there are some tumor cell lines with one-point mutation on *p53* gene. For example, HSC-4 mutant has G to A conversion at the 957th nucleotide, and Ca9-22 mutant has C to T conversion at the 956th nucleotide. First, we prepare three kinds, native HSC-4 and Ca9-22, of fluorescent-labeled anti-sense oligo-DNA. Each FITC-labeled oligo-DNA was hybridized with three kinds of probe-on-carriers and remaining fluorescence with each probe-on-carrier was observed.

As shown in figure 2, the probe-on-carrier carrying native sequence hybridized with the anti-sense of native well, but not with anti-sense of mutated sequence, even though only one-point conversion in those sequence. The probe-on-carriers with mutations also showed best hybridization with their anti-sense probes. This result clearly demonstrated that the oligonucleotide immobilized on porous

glass, probe-on-carrier, acted as a probe to detect for one nucleotide substitution, and may be use to manufacture oligo-DNA array.

3.2 Fixed probe-on-carriers as oligonucleotide microarray

Next step, we tried to make oligo-DNA arrays by the fixation of probe-on-carriers. Immobilization of oligonucleotide probes on solid phase was achieved with a polybutadien adhesive. Off course we checked the adhesive has no fluorescence. The protocol of the hybridization experiment is similar to before. We made fixed probe-on-carriers, native, HSC-4 and Ca9-22 on a slide glass. Then, each fluorescent-labeled anti-sense oligo-DNA was pore on and hybridized with a fixed probe-on-carriers. Remaining fluorescence with each probe-on-carrier was observed after stringency wash.

As shown in figure 3, the fixed probe-on-carrier carrying native sequence hybridized stronger with the anti-sense of native well than with the anti-sense of mutated sequence. The fixed probe-on-carriers for HSC-4 and Ca9-22 also showed the best hybridization with their anti-sense probes, resulting that one point mutation of the

p53 gene could be distinguished with using probe-on-carriers. Therefore, we conclude that the fixed probe-on-carriers may act as an oligo-DNA array. However, the higher background of hybridization spoiled the signal-noise ratio of oligo-DNA arrays. The higher background may be caused by insufficient wash of non-specific hybridization. We must improve the condition of hybridization.

4. Conclusion

Fixed probe-on-carriers are cost effective method to manufacture oligo-DNA arrays, because it dose not need photo mask and chemical modification. Moreover, presynthesized-oligonucleotides on various solid phases are usually stable and could be stocked for longtime. Therefore, probes on DNA chips can be designed by order of the request easier, used for clinical diagnosis freely. Probe-on-carriers are outstanding materials to manufacture oligo-DNA array. We hope probe-on-carriers will contribute for evidence based medicine.

Acknowledgements

This work was partly supported by a Grant-in-Aid for the Creation of Innovations through Business-Academic-Public Sector Cooperation from the Ministry of Education, Science, Sports, Culture and Technology, a grant from the National Center of Neurology and Psychiatry of the Ministry of Health, Labour and Welfare, Japan, and Center for Nano Materials and Technology, JAIST.

Figure legends.

Figure 1. Pore distribution of porous glass

Typical pore-size distributions of 5 different porous glasses, measured by mercury porosimetry.

Figure 2. Result of hybridization with probe-on-carriers.

Porous glasses with different pore-diameter, 30nm and 50nm, were used for the probe-on-carriers. The probe-on-carriers for wildtype and mutant *p53* genes were applied hybridization with fluorescent-labeled oligonucleotides of each *p53* gene. Sequence specific hybridization was studied by remaining fluorescence.

Each probe-on-carrier, column, was hybridized with 3 kinds of fluorescence-labeled anti-sense oligonucleotides, row. Numbers under photographs are relative brightness. 0 means black and 100 means white. Scale bars indicate 100 μm.

Figure 3. Result of hybridization with fixed probe-on-carriers on oligo-DNA arrays

Three oligo-DNA arrays on which three probe-on-carriers, native, HSC-4 and

Ca9-22, fixed were hybridized with each fluorescent-labeled anti-sense oligo-DNA. Remaining fluorescence with each probe-on-carrier was observed after stringency wash.

Numbers under photographs are relative brightness. 0 means black and 100 means white (probe-on-carrier; column, fluorescence-labeled anti-sense oligonucleotides; row, respectively). Bars indicate 100 μm.

References

1. Lipshutz RJ, Fodor SP, Gingeras TR, Lockhart DJ. (1999) High density synthetic oligonucleotide arrays. *Nat Genet*. **21(Suppl 1)**, 20-24.

2. Hacia JG. (1999) Resequencing and mutational analysis using oligonucleotide microarrays. *Nat Genet*. **21(Suppl 1)**, 42-47.

3. Okamoto T, Suzuki T, Yamamoto N. (2000) Microarray fabrication with covalent attachment of DNA using bubble jet technology. *Nat Biotechnol.* **18**, 438-441.

4. Nagasawa H, Matumoto Y, Oi N, Yokoyama S, Yazawa T, Tanaka H, Eguchi K. (1991) Effects of pore size on the retention time of octadescyl silanaized porous glass in high performance liquid chromatography. *Anal sci*. **7 (Suppl)**, 181-182.

5. Tanaka H, Yazawa T, Eguchi K, Nagasawa H, Matuda N, Einishi T, (1984) Precipitation of colloidal silica and pore size distribution in high silica porous glass. *J.Non-Cryst Sol* **65**, 301-309.

6. Maskos U, Southern EM. (1992) Oligonucleotide hydridisations on glass support:a novel linker for oligonucleotide synthesis and hybridisation properties of oligonucleotide synthesed in situ *Nuc Acids Res*, **20**, 1679-1684.

7. Jia L-G, Osada M, Ishioka C, Gamo M, Ikawa S,*et al*. (1997) Screening the *p53* status of human cell lines using a yeast functional assay. *Molecular Carcinogenesis*,**19**, 243-253.

8. Bowtell D, Sambrook J, eds. (2003) DNA Microarrays: A Molecular Cloning Manual. Cold Spring Harbor Press.

Fig.1 Tsukahara and Nagasawa

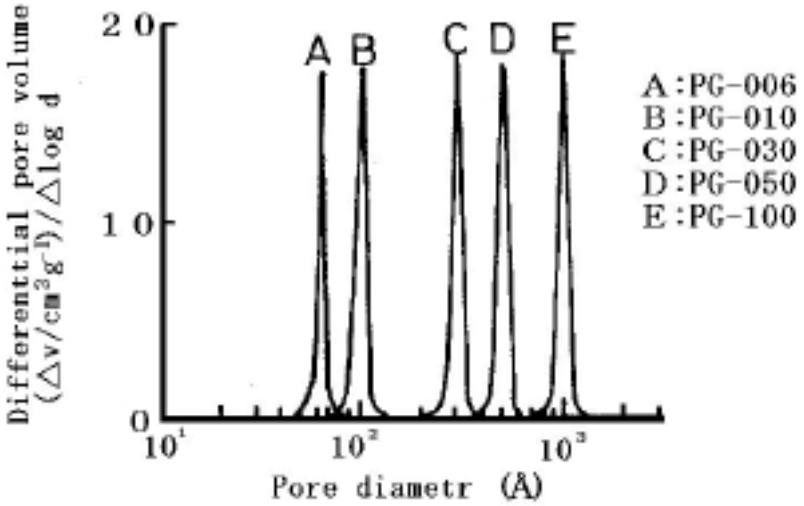

Fig.2 Tsukahara and Nagasawa

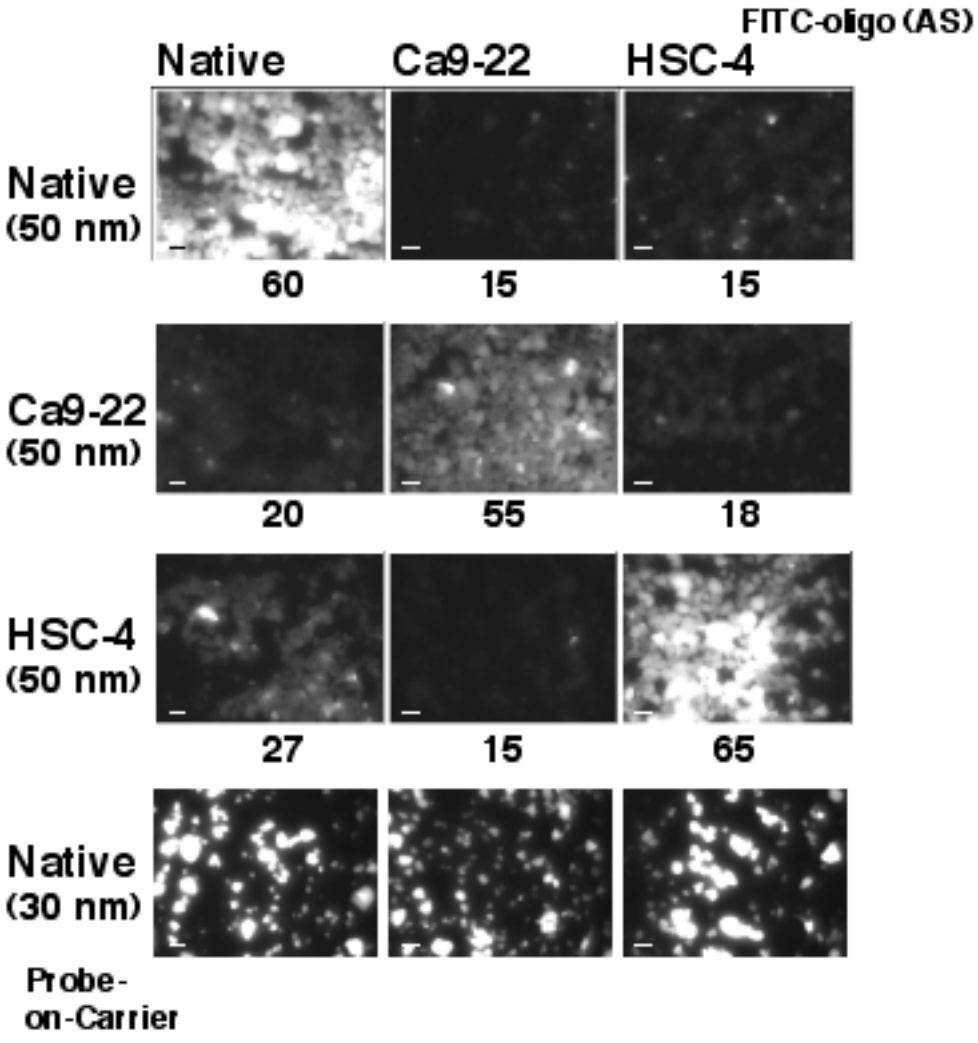

Fig.3 Tsukahara and Nagasawa