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DNA microarrays on ultraviolet-modified surfaces for speciation of bacteria

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ABSTRACT

In this study, we report an approach to activate inert hydrocarbon monolayers with ultraviolet (UV) light to fabricate DNA microarrays. Unlike traditional microarrays that require reactive functional groups on the surface, our DNA microarray is built on an inert layer of *N*,*N*-dimethyl-*N*-octadecyl(3-aminopropyl)trimethoxysilyl chloride silane (DMOAP). This layer is activated by UV (254 nm) just prior to the immobilization of oligonucleotide probes. Our X-ray photoelectron spectroscopy (XPS) results show that new functional groups such as alcohol (C–O), aldehyde (C═O), and carboxylic acid (O—C═O) form on the surface after the UV exposure. Among them, aldehyde groups are responsible for the immobilization of amine-label oligonucleotides. By using this approach, we further optimize UV exposure time and oligonucleotide concentration and also reduce agent concentration to achieve a high density of immobilized oligonucleotides up to 0.16 pmol/mm^2. As a proof of concept, we demonstrate that this microarray can be used for differentiation of different *Clostridium* species such as *Clostridium acetobutylicum*, *Clostridium butylicum*, and *Clostridium beijerinkii*.

DNA microarrays are powerful tools for studying gene expression, single nucleotide polymorphisms, comparative genomic hybridization, DNA methylation, and identification of bacteria [1–5]. Currently, both standard and custom-made DNA microarrays are commercially available from companies such as Affymetrix and NimbleGen [6,7]. However, these chips are very expensive. For example, an Affymetrix chip may cost U.S. $350 to $750, whereas a NimbleGen chip may cost U.S. $200 to $1650, depending on the types of chips and quantities. Meanwhile, each chip often contains a very high density of oligonucleotide probes [8–10]. For instance, PhyloChip from Affymetrix consists of 500,000 oligonucleotide probes capable of identifying 8743 strains of bacteria and Archaea [11,12]. Applications of PhyloChip include rapid profiling of microbial populations in air, water, and soil samples. Most known bacteria can be detected in samples without culturing. Therefore, this is a very powerful technology.

On the other hand, for more specialized applications (e.g., detection of a particular strain of bacteria in food samples), a full-scale DNA microarray is not necessary [13–15]. In these cases, DNA microarrays are often prepared by immobilization of synthetic oligonucleotide probes on glass slides. For example, glass slides can be functionalized by using organosilanes containing reactive functional groups such as aldehyde or epoxy groups to react with amine-terminated oligonucleotides. Glass slides can also be functionalized with amines to react with carboxylated or phosphorylated oligonucleotides. There are already many studies focusing on this area. For example, Dawson and coworkers studied the immobilization of aminated oligonucleotides on aldehyde-decorated surfaces to build DNA microarrays [16]. Lee and coworkers functionalized glass slides with 3-glycidoxypropyltrimethoxysilane with a series of treatments to introduce aldehyde groups on the surface. They also tested their microarrays with murine genes [17]. Wang and coworkers built a microarray on a surface functionalized with epoxy groups and used it to analyze predominant human intestinal bacteria [18]. Moreover, Pack and coworkers investigated binding of oligonucleotides to amine-terminated surfaces [19], whereas Chiu and coworkers studied the combination of amine and epoxy groups on a surface for building DNA microarrays to improve immobilization of oligonucleotides [20]. A common element in these studies mentioned above is that bifunctional organosilanes are required. However, it is more difficult to control the reaction of these bifunctional organosilanes on the surface to form a monolayer.

In this study, we aimed to develop a DNA microarray on glass slides coated with *N*,*N*-dimethyl-*N*-octadecyl(3-aminopropyl)

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Table 1
Sequences of oligonucleotides used in DNA microarray.

Oligonucleotide	Reference bacterium/bacteria	Sequence (5′–3′)
P1	NA	AmC_6F-AACTGGAGGAAGGTGGGGATGACGT-Cy3
P2	NA	AACTGGAGGAAGGTGGGGATGACGT-Cy3
P3	NA	AmC_6F-$(T)_{15}$AACTGGAGGAAGGTGGGGATGACGT-Cy3
T1	NA	Cy5- ACGTCATCCCCACCTTCCTCCAGTT
P4	All bacteria	AmC_6F-$(T)_{15}$CTTGTACACACCGCCCGTCACACCA
P5	All bacteria	AmC_6F-$(T)_{15}$GGAGGAAGGTGGGGATGACGTCAAA
P6	*C. acetobutylicum*	AmC_6F-$(T)_{15}$AGCGGTCGAAGGTGGGGTTGATAAT
P7	*C. acetobutylicum*	AmC_6F-$(T)_{15}$TGGGGACAAAAAGATGCAATACCGC
P8	*C. butylicum*	AmC_6F-$(T)_{15}$AATTACTCTGTAATGGAGGAAGCCA
P9	*C. butylicum*	AmC_6F-$(T)_{15}$ATGAGATGCAACCTCGCGAGAGTGA
P10	*C. beijerinkii*	AmC_6F-$(T)_{15}$AATTACCCTTAATCGGGGAAGCCCT
P11	*C. beijerinkii*	AmC_6F-$(T)_{15}$GTACAGAGAGATGCTAAACCGCGAG
P12	*E. coli*	AmC_6F-$(T)_{15}$CTTCGGGAGGGCGCTTACCACTTTG
P13	*E. coli*	AmC_6F-$(T)_{15}$GGACCTCATAAAGTGCGTCGTAGTC

Note: NA, not applicable.

trimethoxysilyl chloride silane (DMOAP).[1] This molecule contains only a single reactive silane group. Unlike other organosilanes that are insoluble in water, DMOAP contains a positive charge such that it is soluble in water and forms a monolayer on the glass slide very quickly. However, the DMOAP coating layer is inert, and it needs to be activated prior to the immobilization of oligonucleotides to provide reactive functional groups. Furthermore, DMOAP is known to influence orientations of liquid crystals supported on the surface, making the DMOAP-coated slide an ideal substrate to develop liquid crystal-based bioassays. To build a DNA microarray on the inert DMOAP surface, we studied effects of ultraviolet (UV) exposure time, reducing agent concentration, and oligonucleotide concentration on the immobilization of oligonucleotide probes and DNA hybridization. As a proof of concept, optimized DNA microarrays with high density of oligonucleotide probes were further used to differentiate environmental samples containing different species of *Clostridium*.

Materials and methods

Materials and reagents

DMOAP, purpald, sodium hydroxide, sodium cyanoborohydride, sodium borohydride, hydrochloride acid, 2-(*N*-morpholino)ethanesulfonic acid (Mes), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and *N*-hydroxysulfosuccinimide (NHS) were purchased from Sigma–Aldrich (Singapore). Glass slides were purchased from Fisher (USA). Oligonucleotides (Table 1) were custom synthesized by Sigma–Aldrich. 20× SSPE (saline–sodium phosphate EDTA) buffer, 20× SSC (sodium chloride–sodium citrate) buffer, 10% SDS (sodium dodecyl sulfate), and Tris buffer were purchased from 1st BASE (Singapore). 50× Denhardt's solution and deionized formamide and bovine serum albumin (BSA) were purchased from Invitrogen (Singapore). All solvents used in this study were either analytical or high-performance liquid chromatography (HPLC) grade. Reinforced Clostridial Medium (RCM) was purchased from Oxoid (UK).

Surface modifications

To clean the surface, glass slides were immersed in a 5% Decon-90 solution (a commercially available detergent) for 12 h. Then, they were rinsed with an equal volume of deionized water five times and cleaned in an ultrasonic bath twice, each time for 15 min. After this, the slides were dried under a stream of nitrogen. The cleaned glass slides were immersed in a 50 ml of aqueous solution containing 0.1% (v/v) DMOAP. After 5 min, they were rinsed with an equal volume of deionized water five times and dried under a stream of nitrogen. To cross-link the immobilized DMOAP, the slides were placed in a vacuum oven at 100 °C for 15 min. In the final step, the slides were placed 3 cm below a UV lamp (254 nm, Sigma–Aldrich, model 11SC-1) to activate the surface (3 cm was fixed to avoid the effect of heat generated by the UV lamp and the diffusion of oxygen ions).

Surface characterization

X-ray photoelectron spectroscopy (XPS) was obtained by using an AXIS-His spectrometer (Kratos Analytical, Japan). More details of this analysis can be found in our previous study [21]. An aldehyde test was performed on a thin layer chromatography (TLC) plate because the white background of the TLC plate makes it convenient to observe the color change. DMOAP solution was dropped on the TLC plate and dried at room temperature for 10 min. Purpald was dissolved in a 1.0-M sodium hydroxide solution to a final concentration of 2% (w/v). The purpald solution was dropped onto the DMOAP–TLC surface and left for 5 min at room temperature. Then, the color change was observed with the naked eye. A purple color indicates the presence of aldehyde on the surface.

Immobilization of oligonucleotides on glass slides

Oligonucleotides were first dissolved in 3× SSC buffer (pH 7.8) containing 5 mM sodium cyanoborohydride as a reducing agent [22]. Then, the oligonucleotide solution was spotted on a glass slide in an array format by using a spotting robot (AD1500 from Biodot, USA). Each spot contained approximately 100 nl of oligonucleotide solution. The humidity was maintained at 90% throughout the printing process. Then, glass slides were kept in a humid chamber for 18 h, allowing the covalent immobilization of oligonucleotides on the surface. After immobilization, glass slides were washed by using a four-step cleaning process as follows [16]: (i) in 4× SSPE buffer containing 0.1% SDS for 5 min, (ii) in 4× SSPE buffer for 5 min, (iii) in deionized water for 5 min, and (iv) in hot water (90–100 °C) for 10 min. Finally, the slides were dried under a stream of nitrogen.

Reaction of oligonucleotides on DMOAP-modified surface

Reaction of oligonucleotides on the surface was tested by treating the UV DMOAP-modified surface for 20 min with sodium borohydride

[1] *Abbreviations used:* DMOAP, *N,N*-dimethyl-*N*-octadecyl(3-aminopropyl)trimethoxysilyl chloride silane; UV, ultraviolet; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; NHS, *N*-hydroxysulfosuccinimide; SSPE, saline–sodium phosphate EDTA; SSC, sodium chloride–sodium citrate; SDS, sodium dodecyl sulfate; BSA, bovine serum albumin; RCM, Reinforced Clostridial Medium; XPS, X-ray photoelectron spectroscopy; TLC, thin layer chromatography; rRNA, ribosomal RNA; PCR, polymerase chain reaction.