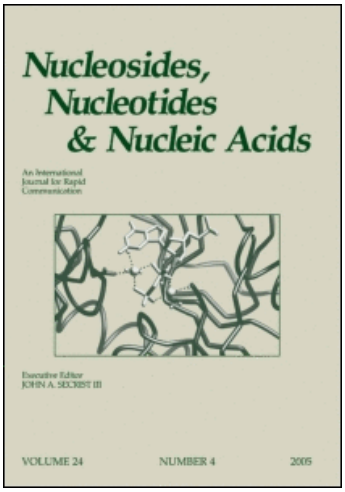

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Jacqueline Fidanza[a]; Marc Glazer[b]; Daniel Mutnick[a]; Glenn McGall[a]; Curt Frank[b]
[a] Affymetrix, Inc., Santa Clara, California, U.S.A. [b] Stanford University, Stanford, California, U.S.A.

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HIGH CAPACITY SUBSTRATES AS A PLATFORM FOR A DNA PROBE ARRAY GENOTYPING ASSAY

Jacqueline Fidanza,[1,*] Marc Glazer,[2] Daniel Mutnick,[1] Glenn McGall,[1] and Curt Frank[2]

[1]Affymetrix, Inc., Santa Clara, California
[2]Stanford University, Stanford, California

ABSTRACT

Colloidal silica particles were deposited on a glass substrate to produce high-capacity porous supports for high-density DNA probe arrays. Porous surfaces were used to increase the addressable surface area and number of probes available for hybridization. Surfaces derived from 70-100 nm size particles deposited in films from 0.15 to 2 microns thick exhibited excellent performance in light-directed oligonucleotide synthesis. Evaluation of these substrates in a genotyping assay is reported.

The rapid and accurate evaluation of large amounts of sequence information made available by the human genome project has become an arduous task. Massively parallel nucleic acid sequence analysis for a variety of gene detection and sequence analysis assays (1–5) has been enabled by high-density polynucleotide probe arrays. Fabrication of high-density DNA probe arrays employs spatially-addressable light-directed combinatorial synthesis (6–7). Probe arrays synthesized in this manner are comprised of short DNA sequences, 20–25 nucleotides in length, that are capable of hybridizing to labeled complementary DNA or RNA target sequences. The hybridized array is either then scanned directly or treated with a stain for signal amplification prior to detection using scanning confocal microscopy. Sequence analysis or gene detection can be determined of the by location of the hybridization reaction on the surface.

*Corresponding author.

A number of methods have been explored to increase the addressable surface area for use in biopolymer arrays (8–11). An increase in the number of probes available for interaction with target should facilitate more hybridization events for greater signal output and for greater sensitivity. These factors are especially important in gene expression monitoring assays where genes in a sample of interest are present in very low copy numbers or where the amount of material for analysis (sample size) is limited.

High capacity supports created by the deposition of colloidal silica solutions onto a glass substrate have been reported (10–11). Porous glass supports created in this manner can be derived from pure silica spheres or can be templated by using silica-latex mixtures. Alteration of the colloidal silica solution composition and/or the deposition process modulates the characteristics of the resultant porous surface. These high capacity supports increase the available surface area in a given two-dimensional lateral space which will be dependant on particle size, particle type and layer thickness. In surfaces comprised of purely silica spheres, the resultant pore size will depend on particle size and packing; and this will ultimately effect the accessibility of the biomolecules within the matrix. Templated surfaces will offer additional flexibility for film morphology in the creation of high capacity surfaces with even larger pores and greater surface area for a given thickness. High capacity substrates derived from both of these methods are currently under investigation for the use as supports for synthesis of DNA probe arrays.

For these studies, porous surfaces comprised of 70–100 nm size particles deposited in films from 0.25 to 0.40 microns thick were utilized. The colloidal silica solutions were deposited by spin coating onto a glass substrate to create chemically and mechanically stable high capacity surfaces. All surfaces were analyzed and characterized both compositionally and functionally as previously reported (11). The surfaces generated in this manner were then silanated to provide sites for probe synthesis (12). A porous matrix composed of approximately 70–100 nm particles should allow for penetration of biomolecules of interest through the matrix. Porous surfaces created in this manner support light directed-DNA synthesis and yield increased hybridization signal in a manner that is proportional to the increased surface area.

MATERIALS AND METHODS

Glass substrates (US precision glass or Hoya) were cleaned prior to use (12). Snowtex-ZL (70–100 nm) colloidal silica solution (Nissan Chemicals) was prepared as a 20 weight percent solution in water, filtered through a 0.45 micron filter and immediately spin-deposited onto the substrate to apply a thin film. Coated substrates were annealed at 350°C for 4 hours (Lindberg Blue M box furnace) then silanated (12) with 1% bis (2-hydroxyethyl)-3-aminopropyltriethoxysilane (Gelest Inc.).

Surface characterization, film thickness, porosity, and surface area were determined as reported (11). All Probe arrays were synthesized using Affymetrix synthesizers and deprotected as described (12).

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Quantitative evaluation of the surface uniformity, surface hydroxyl density and coupling efficiency on the porous silica substrates was conducted using methods based on surface fluorescence (12) and quantitation of surface chemistry (11). Functional performance based on hybridization assays was performed on slides placed in 10 mls of 10–50 nM 5′-fluorescein-labeled complementary oligonucleotide in hybridization buffer (100 mM MES, 1M Na^+, pH 6.6) at 25–45°C with stirring. The surface fluorescence was measured and imaged at various time points. Hybridization assays on packaged GeneChip® arrays were performed according to the recommended Affymetrix, Inc. protocol (13) for the array except where noted.

RESULTS

Figure 1 depicts a SEM image of a high capacity substrate prepared by spin coating a 20 wt.% colloidal silica solution on a glass surface. The resultant surface is a disorganized matrix that contains a considerable amount of polydispersity. Films prepared in this manner exhibit excellent chemical and mechanical stability (11) and there is no significant scattering of the light during chemical synthesis or scanning on these porous matrices. To assess the functional capability of porous silica surfaces to support array synthesis under standard assay conditions, a 20-mer probe was synthesized on the porous surface and hybridization carried out for 16–18 hour at 45°C in MES buffer with 10 nM fluorescein labeled target (11). The observed hybridization enhancement as reported in Table 1, on the porous surface relative to the flat surface indicates that the target can migrate through the porous matrix to hybridize effectively.

For functional assessment in a genotyping assay, a similar coating was used to synthesize a test array comprised of probes representing the HIV protease and

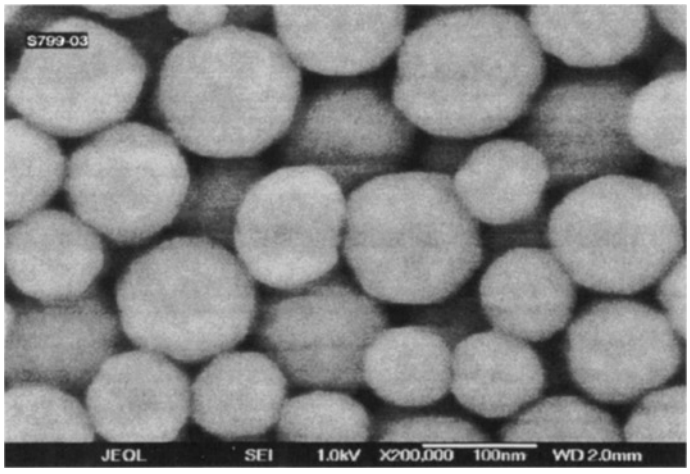

Figure 1. SEM image of the porous glass substrate derived from deposition of 70–100 nm sized silica particles in solution.

Table 1. Hybridization to Colloidal Silica Surface (10–11)

Film Thickness	0.16 um
Estimated Surface Area as Increase Over Flat Glass	7 ± 1
Observed Hybridization Enhancement	6 ± 1

Values are normalized to flat glass.

reverse transcriptase genes. The surface thickness was measured to be about 0.3 microns, which based on the results in Table 1 and on calculations for this film should yield an increase in surface area of 12–18 fold that of a flat surface.

The design format of this HIV array is a sequence analysis tiling (5,13–14) that is performed with a short hybridization time, as target concentrations are not limiting. Targets may be labeled with either fluorescein (13–15) for direct detection, or with biotin for detection via signal amplification (15). A representative HIV assay (13–14) was performed on fragmented fluorescein-labeled HIV cRNA target at 30°C with mixing of the target over the array for 0.5 hour. In this experiment only a selective, but representative portion of the tiled HIV sequence was selected to evaluate target specificity on the porous surface. Overall array performance and the resultant analysis was not evaluated using all of the standard Affymetrix, Inc. GeneChip® tools and algorithms for complete sequence analysis. The HIV target sequence used is in this experiment was not a perfect match to the probe array and thus accounts for sequence call concordance of less than 99% on the standard flat substrate; however, this sequence is acceptable for comparison of high capacity and standard flat substrate arrays. After washing and scanning, the porous surface exhibited a 2-fold increase in signal and slightly greater base call concordance than obtained on a flat surface in the same assay as plotted in Figure 2.

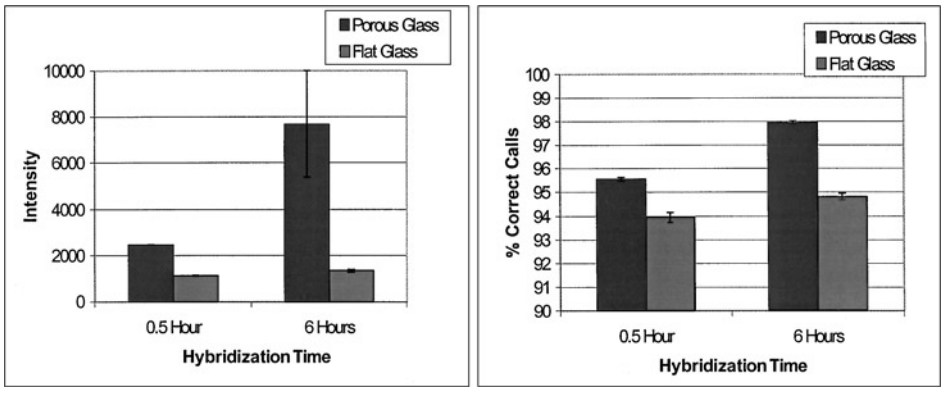

Figure 2. Data from a representative portion of the HIV sequence in a genotyping array. Under standard assay conditions (0.5h hybridization to RNA target at 30°C) the porous glass surface yields a 2-fold increase in signal and enables more concordant calls to be made. The porous surface takes longer to reach equilibrium (approximately 6h) but will yield an 8–10 fold increase in signal.

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The increase in time to equilibrium between the probe and target is due to both mass transport limitations in solution as well as the time for the target to diffuse through the matrix. Consequently, for this experiment the surface was scanned at regular intervals until equilibrium was reached at about 6 hours. At this time an 8-fold increase in hybridization signal intensity over that obtained on flat glass was observed. This 8-fold increase in signal over the flat surface is close to the value anticipated based on surface area. Fragmented RNA target can indeed access the probe sites within the porous matrix and RNA fragments or other reagents do not get trapped within the matrix as there is no increase signal to noise nor is there reduced discrimination by the probes for the target.

CONCLUSIONS

Substrates comprised of colloidal silica can be utilized to create high capacity substrates that effectively support synthesis of high-density DNA arrays. In complex assays, porous substrates hybridized to fragmented labeled RNA to yield a 2-8-fold increase in signal. This demonstrates that biologically derived complex target can diffuse through the matrix and the hybridization signal enhancement attained with this target agrees with that obtained from experiments utilizing complementary oligonucleotides. The HIV assay performed on the porous surface enabled better detection of target and more concordant calls to be made. Furthermore there is no increase in nonspecific binding of target to the array nor are materials trapped within matrix of these high capacity substrates. Studies on kinetics of hybridization within these the porous surface as well as gene expression monitoring experiments are ongoing and will be reported.

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