INTRODUCTION

Gene expression analysis using DNA microarrays [1] has become a standard tool for profiling disease states and monitoring efficacy of therapeutic agents. Typical spotted microarrays have relatively large oligonucleotide spots measuring 100-200 μm, so a reduction in spot diameter presents an opportunity to reduce sample volume requirements, improve binding kinetics, and possibly even eliminate the need for sample amplification. We investigated the feasibility of printing oligonucleotides onto a functionalized surface using the Nano eNabler™ system [2,3].

DATA SUMMARY

Oligonucleotides were designed such that the forward sequence was 5' labeled with fluorescein and the reverse sequence was 5' biotinylated. The first set of oligonucleotides, *forw 1* and *rev 1*, were 37 bases long and the second set, 36 bases in length (Sigma Genosys). Oligonucleotides were diluted in sciSPOT-AL (Greiner Bio-One) to a final concentration of 50 μg/ml. A single lever surface patterning tool (SPT™ print cartridge, catalog # SPT-S-C30) was pre-treated with UV and ozone (ProCleaner™, BioForce) for 30 min and back loaded with diluted *forw 1*. Oligonucleotide arrays (3 X 3) with 20 μm pitch were deposited onto GAPS™ II coated slides (Corning). The SPT™ print cartridge was removed from the Nano eNabler™ system, cleaned and treated with UV and ozone. The SPT™ print cartridge was back loaded with the second oligonucleotide, *forw 2* and deposited (3 X 3 X 20 μm) beside the *forw 1* arrays. The spot size for both oligonucleotides was approximately 4 μm in diameter. The arrayed slides were left O/N at RT to dry and then baked at 80°C for 30 min to immobilize the DNA on the surface.

Pre-hybridization consisted of one 45 min incubation at 42°C with agitation in sciPROCESS-AL buffer (Greiner Bio-One) plus 1% bovine serum albumin. This was followed by two brief washes in diH_2O (RT with agitation), one quick dip in 100% ETOH and finally drying in a stream of nitrogen. For hybridization, the probe (*rev 1 / rev 2*) was diluted 1:10 in sciHYB buffer (Greiner Bio-One), denatured for 3 min and quick chilled. 2 μl of probe was applied to the slide, covered with a pre-cleaned coverslip and hybridized O/N at 42°C in a sealed humid chamber. *Rev 1* probe was placed on one slide and *rev 2* on the other.

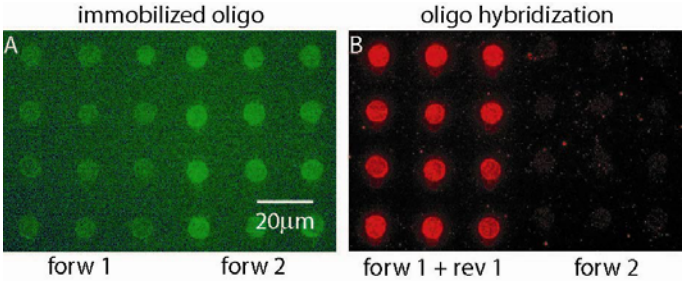

Figure 1. Immobilized and hybridized multiplexed oligonucleotides in ultramicroarrays. Panel A shows the immobilized domains of oligonucleotide *forw 1* and *forw 2*, visualized with a fluorescein 5' tag (green). Panel B shows the hybridization of *forw 1* and *rev 1*, visualized with Streptavidin Alexa Fluor 594™ conjugate bound to the 5' biotinylated *rev 1* (red).

Post-hybridization, the slides were washed 30 min in each buffer, sciWASH I, II and III (Greiner Bio-One) at 42°C with agitation. Streptavidin Alexa Fluor™ 594 conjugate (Molecular Probes) was used to label the hybridized DNA. This was accomplished by diluting the conjugated secondary in 4 X SSC + 0.1% TWEEN 20 and incubating for 1 hour at RT with rotation. The slides were washed 3 X with the same buffer, followed by one brief wash in diH_2O. The signal was examined using a fluorescence microscope and CCD camera (Figure 1).

CONCLUSIONS

The Nano eNabler™ system was used to deposit oligonucleotides in ultramicroarrays with individual domains of 4 μm that are functional and detectable. Potential applications might include ultramicroarray construction or DNA biosensor functionalization.

REFERENCES

[1] Schena, M., Shalon, D., Davis, R.W., and P.O. Brown. Quantitative monitoring of gene-expression patterns with a complementary-DNA microarray. *Science* **270**: 467-470, 1995.

[2] Xu, J., Lynch, M., Huff, J., Mosher, C., Vengasandra, S., Ding, G., and E. Henderson. Microfabricated quill-type surface patterning tools for the creation of biological micro/nano arrays. *Biomedical Microdevices* **6 (2)**: 117-123, 2004.

[3] Xu, J., Lynch, M., Nettikadan, S., Mosher, C., Vengasandra, S., and E. Henderson, Microfabricated "Biomolecular Ink Cartridges" - Surface patterning tools (SPT™ print cartridges) for the printing of multiplexed biomolecular arrays. *Sensors and Actuators B* (in press).