Carbohydrate Microarrays using Arrayjet Non-Contact Inkjet Technology

Printing Versatile High-Resolution Oligosaccharide Microarrays onto a Variety of Substrates Using Arrayjet Microarrayers

Introduction

Oligosaccharides coupled to proteins form conjugates which become versatile reagents for high throughput characterisation of recognition capabilities of monoclonal antibodies, carbohydrate active enzymes, carbohydrate binding modules and other oligosaccharide binding proteins. These microarrays can be successfully printed using Arrayjet non-contact printers onto a variety of substrates. An array library of well characterised plant oligosaccharides has been developed by the Department of Plant Biology and Biotechnology at The University of Copenhagen using Arrayjet robust microarrayers (Pedersen et al., 2012).

Experimental Design

Sample Preparation

Oligosaccharide samples (1→4)-β-D-mannohexaose and (1→5)-α -L-ara binopentaose were prepared by chemical synthesis or hydrolysis of source polysaccharides followed by conjugation reaction to BSA. The samples were dissolved in printing buffer containing 55.2% glycerol, 44% water and 0.8% Triton X 100.

Substrates

A range of substrates were used for oligosaccharide printing including Nexterion® NC, H, P, E, glass slides coated with nitrocellulose (FAST) and nitrocellulose membrane.

Inkjet Printing

Each drop dispensed by an Arrayjet microarrayer is 100 pL. 600 pL spots were printed onto the nitrocellulose membrane. 200 pL spots were printed on to all glass slide types.

Microarray Probing

Following the blocking procedures (Pedersen et al., 2012) nitrocellulose membrane and glass slides were incubated with anti-mannan mAbs- LM21, or anti-arabinan mAb LM6 for 2 hours (1/10 antibody dilution) with PBS and PBS containing 0.05% Tween 20 respectively. All array types were washed with PBS then incubated for 2 hours (1/5000 antibody dilution) with anti-rat or anti-mouse secondary antibodies. Nitrocellulose microarrays were developed using a substrate containing 5-bromo-4-chloro- 3-indolylphosphate (BCIP) and nitrobluetetrazolium (NBT) in BCIP/NBT.

Image Acquisition and Analysis

Nitrocellulose membrane arrays were scanned using a flatbed scanner (Cannon 8800, Søborg, Denmark). The slides were scanned using a slide scanner (GenePix 4100, Molecular Devices, Sunnyvale, USA). The output was analysed using software (ImaGene 6.0, BioDiscovery, ElSegundo, CA, USA).

Results

The images shown in (Figure 1A-F) were obtained using BSA conjugated to (1→4)-β-D-mannohexaose and (1→5)-α -L-arabinopentaose printed in six repeats at a range of concentrations from 2 mg/mL to 0.5 µg/mL (Figure 1A-E) and 2 mg/mL to 30.5 ng/mL (Figure 1F). The most sensitive detection was observed at 2 µg/mL (for (1→4)-β-D-mannohexaose) with Nexterion®E slide (Figure 1D) and the Fast slide (Figure 1E). The least sensitive detection was observed
at 125 µg/mL (for (1→4)-β-D mannohexaose) with Nexterion®P slide (Figure 1C).

In addition, arrays printed on the Fast slide (Figure 1E) produced a consistent spot size across all concentrations and were superior in quality when compared with arrays of Nexterion®E slide. Nitrocellulose membrane arrays produced a sensitive detection at 122.1 ng/mL (Figure 1F). Twelve copies of arrays probed with mAb LM16 and mAb LM21 were printed onto nitrocellulose membrane and FAST slides for reproducibility purposes. Representative replicate arrays are shown in (Figure 2A and B). The mean spot signal from 3 replicate arrays was analysed and data was extracted.

Data Analysis

The data sets from the mean spot signals of arrays (Figure 2A and 2B) were plotted against each other and r2 values were calculated. A low level of variability was observed between the arrays sets with r2 values greater than 0.9 in all cases.

Advantages of Arrayjet Microarrayers

Arrayjet non-contact printers dispense samples by a highly reproducible piezo-actuation process producing quality spots that show constant reproducibility over long print runs and batch-batch. This bears an advantage over pin based contact spotters, where the array quality decreases with longer print runs due to the inevitable wear of the pins with repeated usage (Pedersen et al., 2012).

Arrayjet microarrayers provide flexibility in printing a large number of probes on the same slide with a greater speed. This not only increases the throughput but avoids buffer loss through a concomitant concentration of samples, during longer print runs (Pedersen et al., 2012).

Conclusion

The results of Pedersen et al., 2012 demonstrate that Arrayjet microarrayers can successfully print versatile, high resolution plant oligosaccharides onto a variety of substrates at a fast speed.

Consistent spot morphology, spot reproducibility and signal intensity can be achieved using Arrayjet non-contact printers. This 'on the fly' technology is ideal for printing carbohydrate microarrays and related applications.

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References

McWilliam, I., Chong Kwan, M., and Hall, D. (2011). Inkjet Printing for the Production of Protein Microarrays. In: Protein Microarrays: Methods and Protocols. (U. Korf, ed) Humana Press, New York.

Pedersen, H.L., Fangel, J.U. , McCleary, B., Ruzanski, C., GroRydahl, M.G., Ralet, M-C., Farkas, V., Schantz, L., Marcus, S.E., Andersen, M.C.F., Field, R., Ohlin, M., Knox, J.P., Clausen, M.H., and W.G.T. Willats. (2012). Versatile High Resolution Oligosaccharide Microarrays for Plant Glycobiology and Cell Wall Research. Journal of Biological Chemistry, 287, 39429-38.

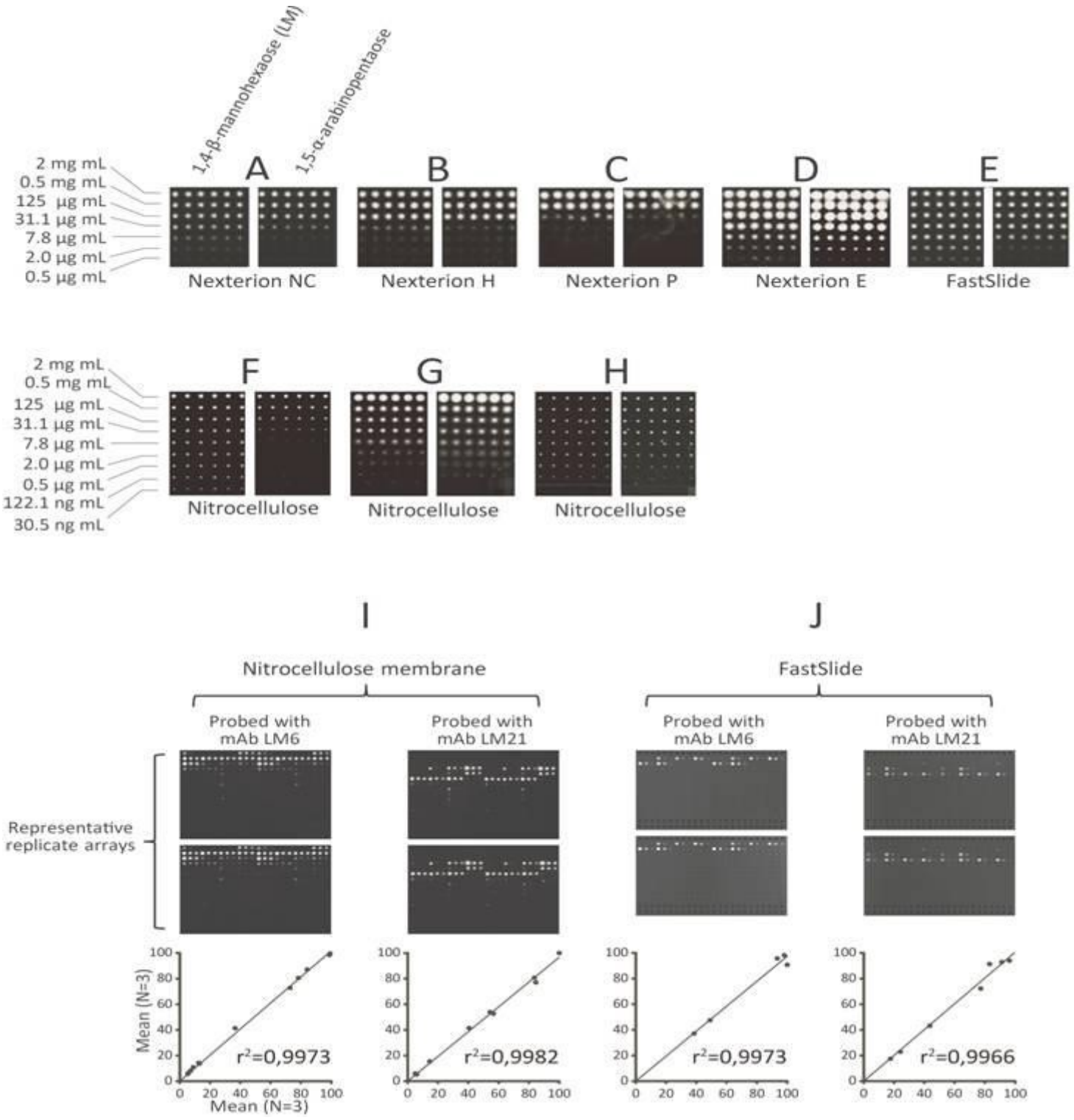

Figure (2): Reproducibility of the microarrays was tested by printing 12 copies of arrays on both nitrocellulose membrane
(A) and nitrocellulose coated glass Fast slides (B). Representative replicate arrays are shown and also graphs of mean spot signals from 3 arrays plotted against each other. Axes on the graphs are relative mean spot signals. r^2= coefficient of
determination, equals to the proportion of variability explained by the linear relationship between X and Y.