sciFLEXARRAYER Application Note
No. 08013

DNA microarrays using sciPOLY3D - Comparison of sciCHIP COP and epoxy-functionalized supports

One major task for the design of microarray based nucleic acid tests is the decision for a platform and substrate material, e.g. polymer microfluidic cartridges, microwell plates, slides or membranes. This choice usually is constrained by the immobilization chemistry. Most conventional immobilization chemistries rely on surface functionalization of the whole substrate with e.g. carboxy or epoxy moieties.
With sciPOLY3D all polymers can be furnished with microarrays without pre-treatment thereof. The only additional process step is a short illumination with UV light after printing. Thus, the whole process of microarray production is reduced to printing and an additional few minutes for irradiation, which can easily be integrated in a high-throughput microarray production line.
The applicability of sciPOLY3D for immobilization of DNA probes on a polymer surface is demonstrated in comparison to sciCHIP EPOXY glass slides. The epoxy chemistry is well established for the immobilization of amine-functionalized DNA probes.

Materials and Methods

Preparation of the sciPOLY3D/DNA microarrays: sciPOLY3D was dissolved in sciPOLY3D SOL1, mixed with sciPOLY3D SOL2D1 and various concentrations of DNA probes. Two kinds of probes were spotted: one oligo with a fluorescent label (Atto647N) for direct observation of immobilization and one unlabeled oligo for observation of hybridization. The mixtures were dispensed with a sciFLEXARRAYER on sciCHIP COP (cyclic olefin polymer) in a microarray format. For comparison: the microarrays on sciCHIP EPOXY slides were spotted with sciSPOT Oligo buffer with various concentrations of amine-functionalized DNA probes. After the spotting process sciPOLY3D microarrays were crosslinked with UV light (1.25 J/cm2 @254 nm) and are immediately ready for use. Microarrays spotted on sciCHIP EPOXY were kept at 75 % rel. humidity for a minimum of 14 hours.

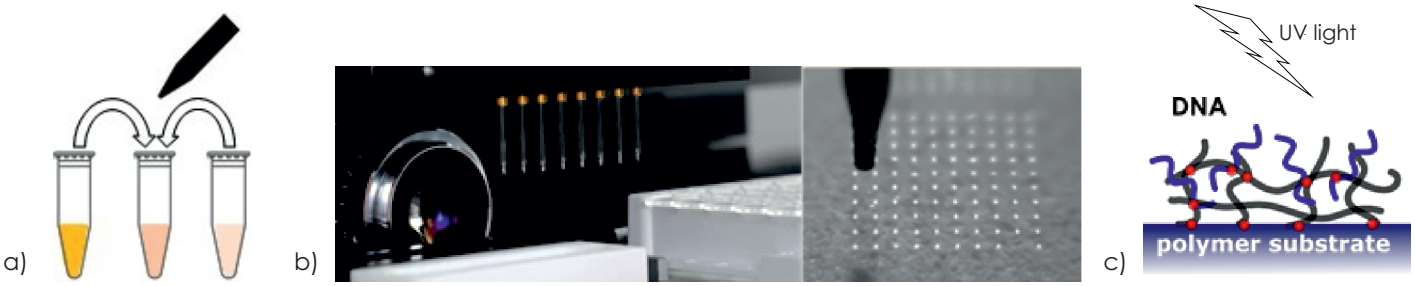

Fig. 1 Manufacturing process of microarrays on unmodified polymer substrates: a) preparation of the mixed solution for printing, b) non-contact dispensing in a microarray format on e.g. polymer slides and plates, c) short UV light exposure to produce covalently attached DNA probes in hydrogel patches.

For testing the hybridization efficiency, antisense DNA oligonucleotides with a fluorescent label (Cy3) were incubated on the different microarrays. The DNA was diluted in sciHYB buffer to a final concentration of 100 nM and incubated using hybridization frames. After washing with sciWASH buffer the microarrays were scanned and analyzed using a GenePix .

Results

As shown in Figure 2, it is possible to tune the density of DNA probes on the surface using both immobilization chemistries. Signal intensities are slightly higher with sciPOLY3D on sciCHIP COP, especially for hybridization (Fig. 2 green). For the highest concentration of labeled probes in sciPOLY3D a slight decrease of the signal was observed, most probably due to quenching of the fluorophore (red).

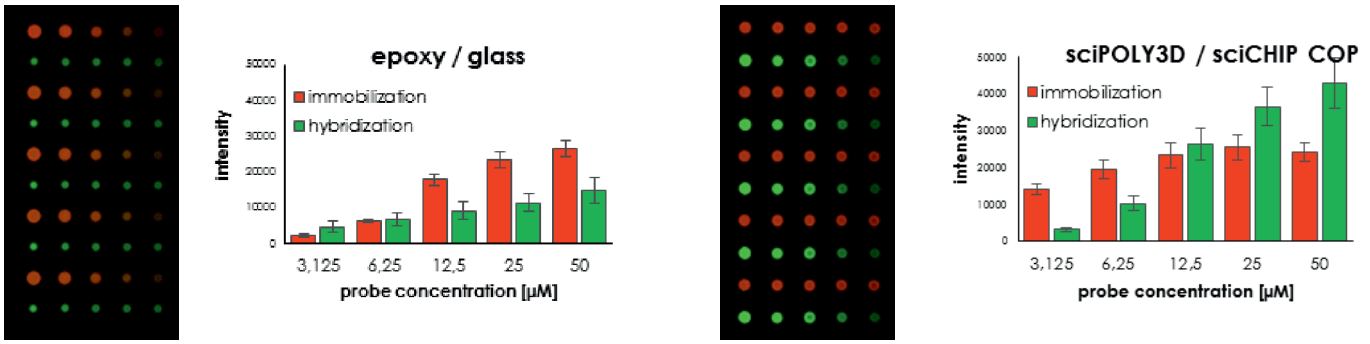

Fig. 2 Comparison of signal intensities of DNA microarrays immobilized on epoxy-functionalized glass slides versus immobilization using sciPOLY3D on sciCHIP COP.

While sciCHIP EPOXY slides are made of glass and require amine-functionalized DNA probes, sciPOLY3D enables microarray production on unmodified polymer substrates with no functionalization required at the DNA probes.

Summary

sciPOLY3D enables covalent and robust immobilization of unmodified DNA oligonucleotides for multiplex detection of RNA and DNA analytes. The use of sciPOLY3D eliminates all wet chemistry steps in the DNA microarray manufacturing process. This feature facilitates the production of microarrays in structured polymer supports such as 96 well plates.

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