sciFLEXARRAYER Application Note
No. 08016

Protein microarrays on unmodified polymer supports using sciPOLY3D

Most conventional immobilization strategies for proteins rely on adsorptive immobilization only or surface functionalization of the whole substrate with e.g. carboxy or epoxy moieties.
With sciPOLY3D proteins can be covalently attached to virtually all polymers without pre-treatment thereof and even on protein-repellent surfaces. sciPOLY3D is water soluble and is simply added to the printing media. 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.

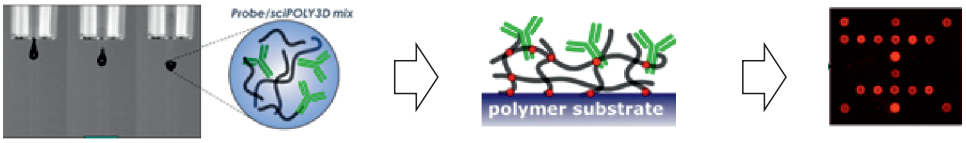

Fig. 1 Manufacturing process of microarrays on unmodified polymer substrates: non-contact dispensing in a microarray format on e.g. polymer slides and plates, short UV light exposure to produce covalently attached probes in hydrogel patches and fluorescence detection

Comparison of sciPOLY3D on a protein-repellent surface with a sciPLEXPLATE Type 1

To compare the performance of covalent immobilization of antibodies on a protein-repellent surface using sciPOLY3D with standard adsorptive immobilization on a sciPLEXPLATE Type 1, an IL-10 sandwich immunoassay was performed. The analyte was applied in a dilution series starting at 2 ng/ml, down to 0.32 pg/ml. The result is shown in figure 2, there is no significant difference in the course of the response to the increasing analyte concentration. With sciPOLY3D a prior blocking step is obsolete.

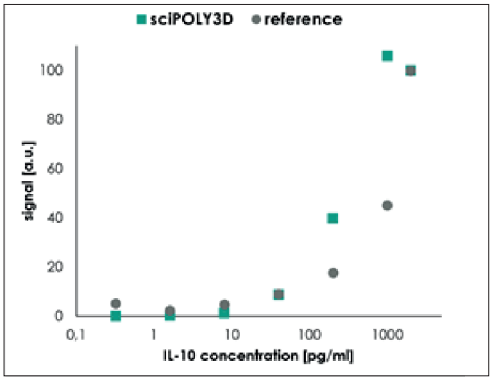

Fig. 2 Comparison of an analyte (IL-10) dilution series processed on microarrays printed with sciPOLY3D on non-binding 96 well plates (green squares) and without sciPOLY3D on sciPLEXPLATES Type 1 (grey circles).

Compatibility with different assay formats: Immobilization of antibodies or antigens

There are two main immunoassay formats commonly used, either with antibodies as capture probe for detection of different kinds of antigens, or vice versa, with antigens immobilized for capturing antibodies. Both formats were tested with sciPOLY3D, as shown in figure 3 and figure 4. Antibodies specific for different cytokines were immobilized, exemplarily shown is the successful detection of IL-6 and IL-10, respectively in a sandwich immunoassay. These arrays were printed on a protein-repellent surface; thus no blocking was performed prior the assay.

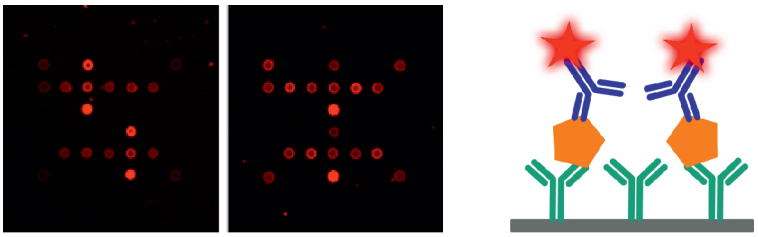

Fig. 3 Sandwich ELISA with immobilized antibodies specific for a panel of cytokines. Exemplarily shown are color-coded fluorescence images after performing assays with samples spiked with IL-6 (left) and IL-10 (middle). On the right hand side, the assay format is depicted schematically.

If antibodies need to be detected, e.g. for allergy tests or autoimmune diagnostics, one has to immobilize antigens with a wide variety of properties or even complex protein mixtures. sciPOLY3D was tested successfully for this challenge. In the figure below the result is shown of an allergy test on different plant and animal allergens.

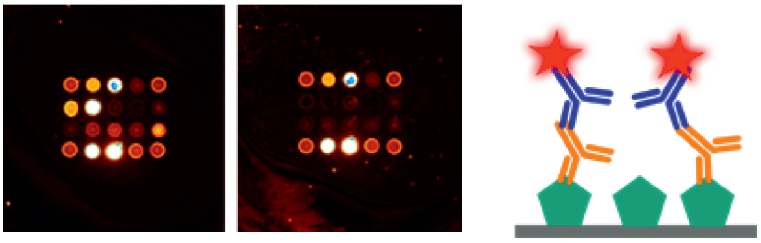

Fig. 4 Allergy test with a microarray containing protein extracts from different plants and animals, immobilized with sciPOLY3D. Exemplarily shown are color-coded fluorescence images after performing assays with positive serum sample (left) and negative serum sample (middle). On the right hand side, the assay format is depicted schematically.

Compatibility with detection formats: Fluorescence and colorimetric

There are different reasons for choosing a certain detection format, like desired sensitivity, available read-out options, etc. With sciPOLY3D there are no restrictions, both fluorescent and colorimetric detection is feasible. In figure 5 two microarrays in a 96 well plate are shown after immunoassay processing, left one was stained with Cy5, right one was stained with HRP and TMB. They both show the same result.

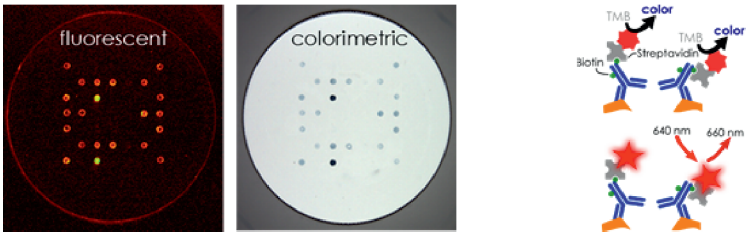

Fig. 5 Two protein microarrays printed with sciPOLY3D in a 96 well plate. They were both processed identically with an IL-6 immunoassay. Left one was stained with Cy5 and detected in a Tecan LS fluorescence scanner, right one was stained with HRP and TMB and detected in a sciREADER CL2 colorimetric reader.

Summary

With sciPOLY3D virtually all common plastic substrates, such as COP, PMMA, COC, PP, etc. and even non-fouling surfaces can be used for microarray applications. This feature eliminates all wet chemistry steps in the microarray manufacturing process. This facilitates especially the protein immobilization on structured substrates, such as microfluidic chips and on substrates that are not suitable for adsorptive immobilization.

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