

PROTEIN MICROARRAY TECHNOLOGY

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INTRODUCTION:

Biochip technology allows the simultaneous analysis of thousands of molecular parameters within a single experiment. Most of the current applications focus on DNA array technology for gene expression analysis or the detection of single nucleotide polymorphism¹. However, any kind of ligand binding assay which relies on the product observation of an immobilized capture molecule and its binding partner from the surrounding solution can be imagined to be performed as an array experiment². Such protein chips will provide a powerful and reliable platform for extending molecular analysis beyond the limitations of DNA chips. In our laboratory we are developing microarray based sandwich immunoassays looking at different types of immunoglobulins. Autoantigen micro arrays are used to screen in parallel for the presence of auto-antibodies from minimal amounts of patient sera. Furthermore, immobilized peptides and proteins can be used to search for their corresponding cell surface receptor on living cells.

METHODS: A GMS417 Arrayer (Affymetrix, USA) was used for the fabrication of the arrays. The capture molecules were diluted in stabilizing buffer containing 20% glycerol in PBS and 5 µg/ml BSA. Silylated slides (TeleChem International, Inc., USA) or Poly-L-Lysine coated slides (Sigma Aldrich; FRG) were used as solid supports. Prior to spotting bromophenol blue was added to the stabilized antigen solutions to a final concentration of 0.1%. This enabled us to control the microspot application and the integrity of each array. Capture molecules on the slides were rehydrated in PBS for 5 min, incubated with blocking buffer (1.5% bovine serum albumin, BSA, 5% low fat dry milk in PBS) for 30-60 min and incubated with 20 µl target diluted in blocking buffer + 0.1% Tween20). Unbound target was washed away. Detection of bound target was performed with either a Cy5 conjugated secondary antibody or for the sandwich immunoassays with a biotinylated secondary antibody followed by a final Cy5 streptavidin incubation. Images of the microarrays were taken with a Affymetrix GMS 418 Scanner.

RESULTS: Our microarray based sandwich assays allows us to determine in parallel IgG, IgA and IgM from minimal amount of sera.

For autoantibody detection involved in Type 1 or insulin-dependent diabetes mellitus (IDDM) GAD65, IA-2, insulin and control proteins were screened simultaneously the sera of individuals. Autoantibodies present in just a few µl of serum can be detected via a micro-ELISA by fluorescence measurements.

Cell based microarray have been developed that can be used to screen for peptides that improve specific cell adhesion. Laminin peptide sequences with different spacers were covalently bound to the arrays. On these coated solid supports, tectum cells (chicken) were cultivated for 24 hours. The cells adhering to the single spots were made visible using a DAPI dye. Cells were found only on those surfaces coated with the adhesion mediating peptide. The evaluation of the average spot intensity offers an easy and efficient method to elucidate varying cell adhesion on different surfaces quickly and reproducibly.

DISCUSSION & CONCLUSIONS: Microarray Technology allows the simultaneous analysis of a multitude of parameters with a single experiment. Miniaturization and parallelization succeed in increased speed, higher precision and decreased reagent consumption, thereby lowering costs considerably in diagnostics.

REFERENCES: ¹Phimister, B., ed. *The chipping forecast*. supplement ed. **21**. 1999, Nature Genetics, ²Ekins et al., 1989 J. Bioluminescence and Chemiluminescence **4**, 59-78,

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