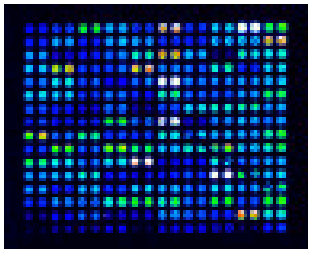
Plasma surface modification of DNA and Protein Microarrays

By Demetrius Chrysostomou, PhD., Director of Technology, PVA TePla America



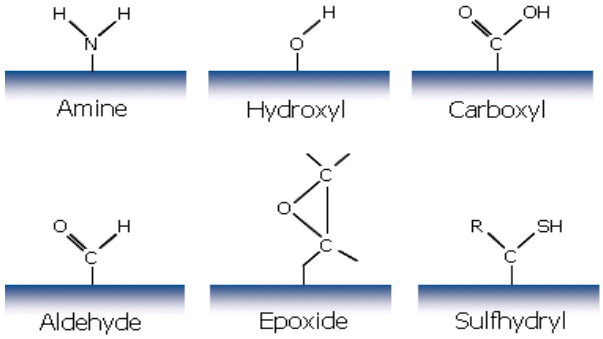
Microarray technology has accelerated the rate of biomolecular research by enabling an enormous number of experiments to be conducted in parallel. This is achieved by immobilizing arrays of probe biomolecules, such as oligonucleotides or proteins, in picomole quantities onto a substrate. The attachment of these probes requires chemically functionalizing the surface. This is a critical step in microarray fabrication and plays a significant role in their functional performance. Plasma surface functionalization reduces the complexity of wet chemical treatments, controlling surface cleanliness, functional chemistry and hydrophobicity in a single, automated process step.

What is plasma?

Plasma is a gas energized to a state of electrical conductivity. Chemically it is a highly reactive environment that is used to control properties of surfaces without affecting the bulk material. This is accomplished by first cleaning the surface at the molecular level. It then activates both the surface and the chemical precursor that is fed into the plasma. Chemical grafting of the precursor to the surface takes place at low temperature in a reliable, consistent, and environmentally friendly manner.

Microarray operating principle

DNA and protein microarrays have fast become essential tools in genetic sequencing, genetic variation analysis, and gene expression. They are used in research fields such as gene discovery, identification of bio-pathways, disease biomarker detection, prediction of drug responsiveness, *etc*. Hybridization/bonding of the surface immobilized probe molecule with a labeled target molecule is the principle behind their operation. In DNA microarrays the higher the number of complementary base pairs between nucleotide strands of the probe and target the better the bonding. After washing only the strongest paired strands remain hybridized. Probe/target bonding is usually quantified photonically via labeled targets.



Above are the functional groups that plasma can modify microarray platforms with. The functional groups are deposited onto surfaces via a PECVD-like process producing plasma polymers with the desired chemical functionality.

Amino and epoxy functionalities

Amino functionality provides positively charged binding sites for the electrostatic attachment of oligonucleotides via their negatively charged phosphate groups. If steric hindrance interferes with direct binding of these large biomolecules, linkers provide space for the biomolecule to adsorb in the right configuration. Linker molecules themselves are anchored to the substrate via plasma chemical grafting e.g. amino groups.

Amino groups increase surface energies rendering them hydrophilic. Overly hydrophilic surfaces may not be desirable when depositing e.g. gel drop arrays onto a microarray platform, because the micro droplets may wet in a malformed manner. Again, gas plasma can solve this problem and preserve the morphology of the droplets by controlling the surface energy, even in the presence of the amino groups.

Epoxy plasma functionalization has proved a successful surface treatment, particularly for protein microarrays. The surface is more hydrophobic compared with other chemical functionalities, facilitating fabrication by reducing spot spreading, and more importantly showing more resistance to non specific adsorption. Non specific adsorption of DNA strands on the sensor surface will increase the background and reduce the selectivity of the sensor.

What are the benefits of plasma surface modification of microarray platforms?

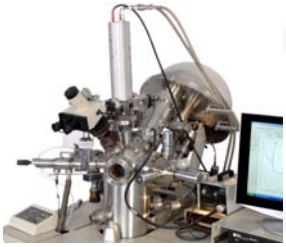
The chemical functionalization of microarray platforms using wet chemistry has observed the

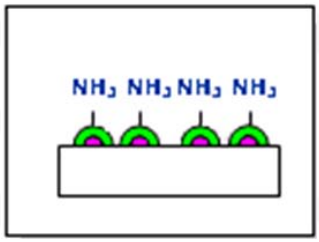
aggregation of functional groups on the surface. Aggregation causes steric hindrance. This problem can be mitigated by combining amino and epoxy functionality. Aggregation of similar functional groups has not been observed using plasma to modify the surface. Indeed plasma treated surfaces are highly conformal, particularly if plasma polymers are deposited. Plasma polymers have the added advantage that they are independent of the chemical properties of the substrate thus adding additional control.

Plasma procedures are a lot less complex than wet chemical, being a single step process that can be performed in a fully automated system. Finally, plasma surface modification is much quicker than wet chemistry; 10 minutes versus some hours.

How does PVA TePla America validate their processes?

X-ray photoelectron spectroscopy (XPS) and surface derivatization techniques are used to quantify the percentage of the surface that has been functionalized with desired chemical groups.





Left: *XPS (Perkin Elmer) with 500mm analyzer*
Right: *Graphic of primary amine functionalized surface*

Case Study

What does PVA TePla America offer?

At PVA TePla America we offer a full line of vacuum and atmospheric gas plasma systems. Our reliable, easy-to-operate products deliver some of the most advanced and innovative solutions for a wide variety of industrial applications. We also offer clean area contract processing services with ISO 9001:2008 certification.

This allows you to access gas plasma technology without up front capital expenditure on labor and/or facilities. Additionally, we offer free proof of process as an incentive to evaluate our plasma technology.

PVA TePla America Inc. Headquarters

251 Corporate Terrace
Corona, CA 92879-6000

www.pvateplaamerica.com

business: 951.371.2500
sales: 800.527.5667
fax: 951.371.9792

Certified System™
ISO 9001
QMI-SAI Global