European Cells and Materials Vol. 10. Suppl. 5, 2005 (page BS10) ISSN 1473-2262

Fundamental DNA-surface analysis to understand microarray assay limitations

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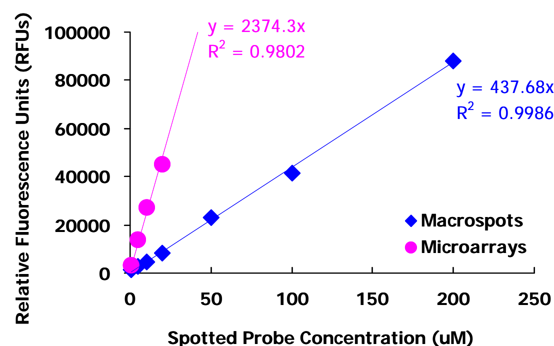
INTRODUCTION: We have characterized amine-derivatized, single-stranded DNA (5'-NH_2-$(CH_2)_6$ CTG AAC GGT AGC ATC TTG AC-3', abbreviated NH_2-Oligo1) attached to amine-reactive commercial microarray slides using X-ray photoelectron spectroscopy, fluorescence imaging and ^{32}P-radioassays. Immobilization efficiencies of NH_2-Oligo1 under microarray format can be reproduced at macroscopic levels for surface analysis using high salt and increased DNA concentrations with good reliability and reproducibility. Hybridization efficiency with complementary DNA (Oligo2) was studied on these surfaces. The macroscopic model provides a platform for study of DNA surface chemistry using highly sensitive, quantitative surface analytical techniques.

METHODS: Two brands of commercial amine-reactive microarray polymer slides were purchased from vendors. (CodeLink™, Amersham, NJ, and OptArray™, Denver, CO). DNA oligonucleotides were purchased from TriLink (San Diego, CA). DNA microarrays were printed using a SpotBot™ microarray printer (TeleChem, Sunnyvale, CA) and hybridized following commercial protocols. For macroscopic immobilization, NH_2-Oligo1 at concentrations ranging from 0 to 200μM in 1M sodium phosphate buffer were applied to commercial slides at 100% humidity. Post-coupling treatment and hybridization were conducted identically to microarray formats. In ^{32}P-radioassay experiments, oligonucleotide probes were labeled with α-^{32}P-ddATP (Amersham Biosciences, Piscataway, NJ) in the presence of terminal transferase[1] (Roche, Indianapolis, IN). Surface reaction procedures were the same as non-radioactive experiments. XPS spectra were obtained using a PHI5800 instrument (Al anode operated at pass energy 58.70eV, 35° take off angle). Fluorescence imaging was performed using a ScanArray Express™ fluorescence scanner at 90% laser power and 45% PMT, with 10μm or 50μm resolution. Phosphor imaging was performed with a Storage phosphor imager™ at 200μm resolution.

RESULTS: We studied the NH_2-Oligo1 immobilization on commercial amine-reactive microarray slides both in microarray and macroscopic format. Ionic strength of immobilization buffer was observed to play a critical role in DNA probe immobilization efficiency. Rapid solvent evaporation in microarray printing procedures result in significantly increased local ionic strength as well as higher DNA concentration. By increasing bulk immobilization buffer salt concentration and DNA concentration, we were able to obtain the same level of immobilization efficiency macroscopically, i.e. spots with diameters of millimeter dimensions. (Figure) Immobilization efficiencies of macro-spots were compared with microarrays using fluorescence imaging. Surface properties of such macro-spots with DNA modified commercial slides were analyzed using XPS and ^{32}P-radioassay. Densities of DNA Oligo1-immobilized surface were found to be at the 10^{12}-10^{13} molecules/cm^2 level and exhibited high hybridization efficiency (70%~100%).[2]



CONCLUSIONS: We have modeled microarray amine-DNA immobilization chemistry and efficiencies at a macroscopic level using high salt buffer and high DNA concentrations. Macro-spots obtained this way provide a good analytical platform for surface analysis of DNA immobilization and hybridization using quantitative surface techniques including XPS and radioisotope labeling.

REFERENCES. (1). Tarlov, M. H. et al, *J. Am. Chem. Soc.* 1997, *119*, 8916-8920. (2). Georgiadis, R. M. et al, *Nuc. Acids Res.* 2001, *24*, 5163-5168.

ACKNOWLEDGEMENTS: The authors acknowledge financial support from NIH EB001473-02, Dr. C. Greef, Dr. M. Lochhead and Dr. W. Hanneman are thanked for insightful discussions and technical assistance.