Investigation of Surface Hydrolysis in Thin Organic Films Using Time-of-Flight Secondary Ion Mass Spectrometry and Principal Component Analysis

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Statement of Purpose: Chemical reactions at organic surfaces have been widely used in array technologies, biosensors, and surface modifications for biomedical applications.[1-3] N-hydroxysuccinimide (NHS) active esters are used to covalently couple amine-terminated biomolecules onto surfaces. Their intrinsic hydrolytic instability is well-known in conjugation chemistry and is a concern for maintaining a stable, reactive surface, especially for storage of the surfaces.[1] Therefore, we investigated the surface hydrolysis in well-defined NHS-ester terminated oligo (ethylene glycol) (NHS-OEG) self-assembled monolayers (SAMs) using Time-of-flight Secondary Ion Mass Spectrometry (ToF-SIMS) and Principal Component Analysis (PCA). The secondary ion fragments of NHS, carboxyl, OEG, hydrocarbon and Au species were identified. Then, a multivariate peak ratio derived from PCA results on the entire spectrum was proposed as a measurement of the NHS surface reactivity (the amount of bound NHS groups available for binding biomolecules).

Methods: Freshly gold-coated wafers were immersed in ethanol solution of NHS-OEG_7 disulfide (Polypure). After disulfide assembly, samples were rinsed thoroughly by ethanol. Surfaces were then blown dry with nitrogen and store under nitrogen until further aging. Samples prepared less than 0.5 h before analysis are denoted "fresh" samples. Surfaces exposed on the lab bench to ambient humidity for 1 hr to 7 days are denoted "aged." A second subset incubated overnight in a 100% relative humidity chamber is denoted "100% rh" A third subset soaked in Millipore water overnight to hydrolyze surface NHS groups was denoted "hydrolyzed." Static positive ion ToF-SIMS data were acquired using a PHI Model 7200 ToF-SIMS instrument. All of the peaks with intensities at least 3 times above background in the 12 – 350 m/z region of the positive ion mass spectra were selected for PCA. A discussion of PCA for ToF-SIMS analysis is available elsewhere.[4]

Results/Discussion: PCA of the positive ion ToF-SIMS data showed that the changes in the outmost surface of the thin film were related to the various sample storage conditions. The first PC captured 60% of the variance in the positive ion SIMS data, indicating the variation was primarily due to the aging conditions. The loadings plot was examined to determine the cause of the sample separation. Intensities of hydrocarbon, carboxyl and Au related peaks increased as a function of sample aging time, whereas intensities of NHS related peaks decreased. The loadings from PC 1 were used to develop a multivariate peak intensity ratio representing NHS reactivity (Equation 1). These ratios were calculated to separate out the aging effects on the positive secondary ion spectra due to NHS reactivity, hydrocarbon accumulation, and the NHS loss. (the PC 1 scores combine these three aging effects). NHS reactivity ratio represents the relative NHS reactivity coverage in the SAMs.

$$NHS\_reactivity = \frac{I_{high-mass\_NHS^+}}{I_{carboxyl^+}}$$

where, $I_{high-mass\_NHS^+} = I_{C_7H_8O_4N^+} + I_{C_6H_8O_3N^+} + I_{C_5H_6O_3N^+}$

$$I_{carboxyl^+} = I_{C_5H_9O_3^+} + I_{C_3H_5O_2^+} + I_{CHO_2^+}$$

Equation 1. NHS reactivity ratio, the summation of three high mass NHS peaks to summation of carboxyl peaks.

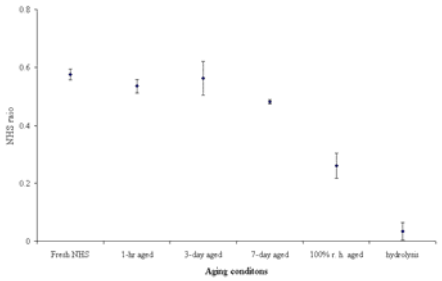

Figure 1. NHS reactivity ratio as a function of treatment condition show the NHS reactivity deceases upon aging.

Conclusions: The secondary ion spectra of NHS-OEG SAMs were significantly affected by the sample storage conditions. A multivariate peak ratio was developed from the PCA results that give insight into the NHS reactivity. This multivariate peak ratio was useful for assessing the ToF-SIMS data from analogous surfaces without requiring additional PCA. The results from these studies are useful for developing improved methods for the storage and regeneration of NHS surfaces.

References:

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Acknowledgment: This research was supported by NIH grants EB-002027 and EB-001473. Special thanks to Dr. Roger Michel (UW) and Dr. Daniel Graham (Asemblon).