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Time-of-flight secondary ion mass spectrometry (TOF-SIMS) for high-throughput characterization of biosurfaces

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Abstract

A graded oxidation process, involving UV-ozone (UVO) treatment, was used to create a poly(ϵ -caprolactone) (PCL) surface with a systematic variation in surface chemistry. Time-of-flight secondary ion mass spectrometry (TOF-SIMS) has proved useful in characterizing the chemical composition of these surfaces and in monitoring the oxidation process. The TOF-SIMS data correlates with contact angle data and the results of the binding studies performed with mouse calvarial cells. UVO treatment resulted in a PCL surface with improved wettability and cellular adhesion.

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1. Introduction

The extension of combinatorial methods to materials science has resulted in a shift in emphasis from screening for “hits” and the design of molecular structures (as in drug discovery) to knowledge discovery obtained by rapidly exploring multivariable-parameter space and investigating how those variables (compositional, processing, etc.) affect phenomenological physics. Polymeric materials are finding new applications in bioreactors, tissue implants and bio-delivery devices. To successfully introduce these into new products, it is imperative to rapidly identify the biocompatibility of newly developed materials,

particularly with the surface treatments adopted. Surface properties, such as surface free energy, are among the key issues influencing biocompatibility. In previous work [1], we presented a novel method for creating surface free energy gradients using UV-ozone (UVO) treatment of silane monolayers. The graded oxidation process resulted in surfaces with a systematic variation in surface chemical composition across the substrate. Time-of-flight secondary ion mass spectrometry (TOF-SIMS) was used to provide information on the surface’s chemical signature. This information was then correlated with the hydrophobicity and hydrophilicity (assessed by way of contact angle measurements) of the surface. In this study, we extend the graded oxidation process to modification of poly(ϵ -caprolactone) (PCL) surfaces and utilize TOF-SIMS in a combinatorial assessment of biocompatibility.

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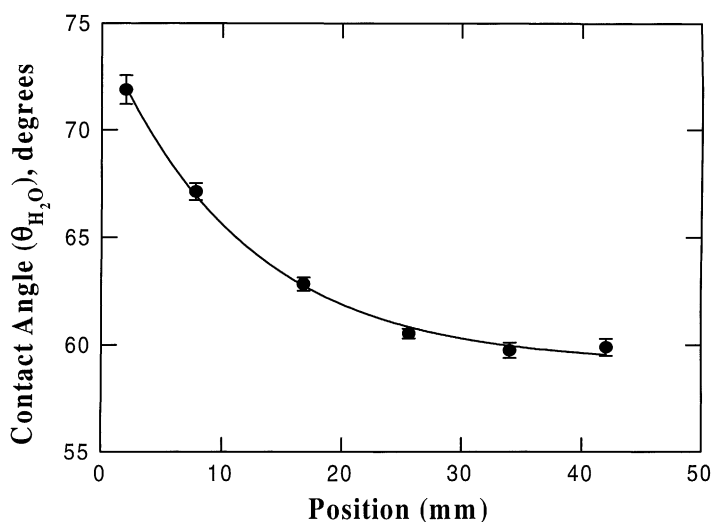


Fig. 1. Contact angle data.

2. Experimental

PCL was obtained from Aldrich Chemical Company¹ ($M_n \approx 80,000$; Lot No. 07526HI; mp 60 °C). A 1% (mass fraction) solution was prepared in toluene from which 50 nm thick films were spun-cast onto 10 cm (diameter) silicon wafers (n-type, $\langle 100 \rangle$, Wafer World Inc., FL) The wafers were then cleaved into $\approx 4.5 \text{ cm} \times 3 \text{ cm}$ rectangular sections. Static contact angle measurements for 1.5 ml drops of water (deionized; resistivity $\approx 18 \text{ M}\Omega$) were performed at room temperature with a Kruss G2 contact angle measuring system. The films were exposed to gradient UVO radiation through a custom-designed variable density filter (Maier Photonics). The filter was placed with a spacing of $\approx 500 \mu\text{m}$ from the surface to allow oxygen to diffuse across the sample. The polymer films were exposed to the UVO treatment in a Jelight UVO Cleaner[®] (Model 42) for 10 min. A low-pressure mercury vapor grid lamp is the UV source in this apparatus. The films were immediately transferred to the SIMS instrument for surface analysis. Positive and

negative static SIMS analyses were performed with the TOF-SIMS IV (Ion-TOF, Münster, Germany). Spectra were generated with a pulsed 25 kV gallium ion beam (25 nA DC current, 20 ns pulse width) and were collected in $500 \mu\text{m} \times 500 \mu\text{m}$ areas along the length of the rectangular sample. Each automated spectral acquisition required 300 s. A pulsed, low energy ($\approx 30 \text{ eV}$) electron flood gun was used for charge neutralization. Automated acquisition of the spectra was achieved by using the Microsoft dynamic data exchange (DDE) protocol from within a Python script to control Ion-TOF's spectral acquisition program and the Raith ESCOSY[®] stage control software. The Python script started acquisition of a spectrum at a particular analysis position, saved it with a unique file name and then stepped the stage to the next analysis position. Forty spectra were collected over a period of 4 h. Once the data were collected, analysis was performed either manually or by means of a custom program. Cell binding studies were performed using osteoblast-like cells in a tissue-culture laboratory.

3. Results and discussion

Fig. 1 shows contact angle measurements for the surface. The contact angle measurements were obtained starting at the most hydrophobic end of the sample; as such, the contact angle plot shows a

¹Certain commercial equipment, instruments, or materials are identified in this paper in order to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best for the purpose.

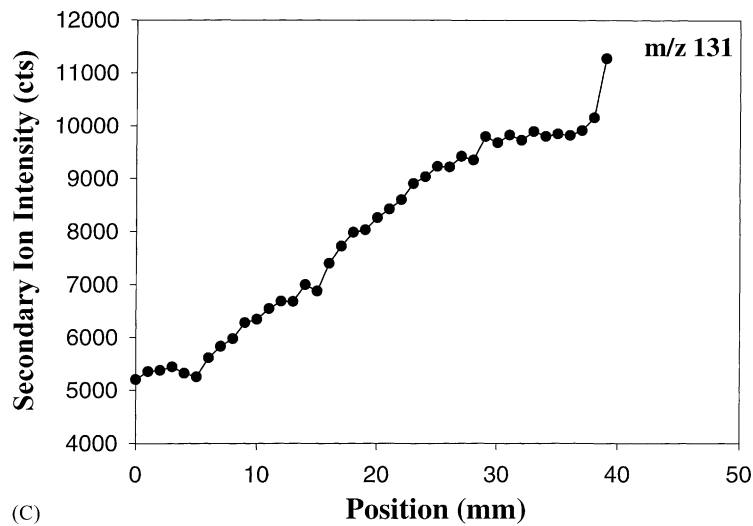
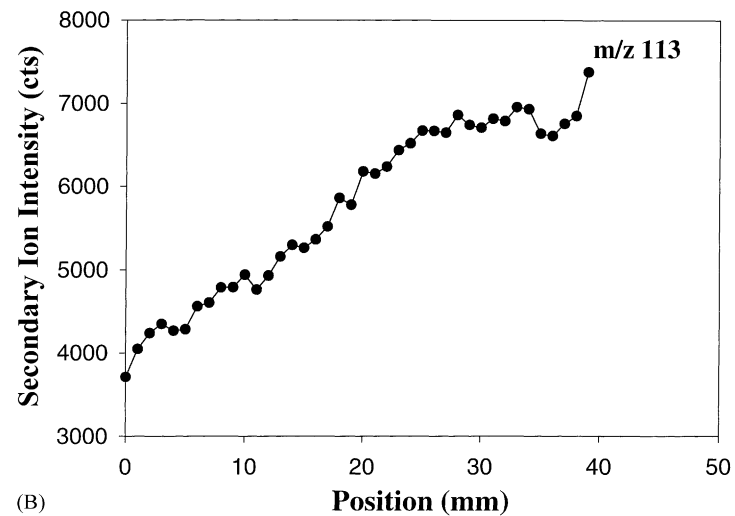
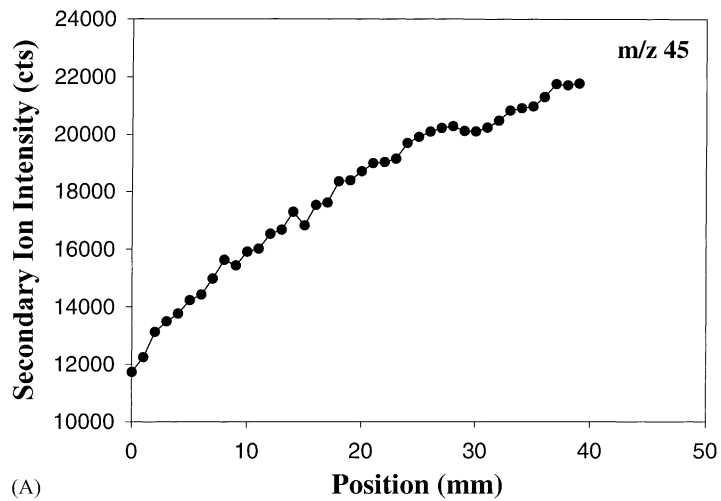


Fig. 2. Negative secondary ion trends observed for (A) COOH^- (m/z 45), (B) $\text{C}_6\text{H}_9\text{O}_2^-$ (m/z 113) and (C) $\text{C}_6\text{H}_{11}\text{O}_3^-$ (m/z 131) along the length of the PCL sample—results for 40 spectra.

steady decrease in θ (increase in surface energy) as the surface becomes more hydrophilic. The error bars in Fig. 1 (evaluated for multiple samples and multiple measurements for each sample) show a standard error less than 1% for identical conditions of sample preparation. Negative ion survey spectra of the surfaces revealed that the most intense characteristic peaks for the PCL surface were at m/z 45 (COOH^-), m/z 113 ($\text{C}_6\text{H}_9\text{O}_2^-$) and m/z 131 ($\text{C}_6\text{H}_{11}\text{O}_3^-$). The intensity of the characteristic ions differed by approximately 45–50% at the hydrophobic and hydrophilic extremes of the sample (data not shown). The film was then subjected to automated analysis. Fig. 2A–C show the secondary ion trends observed for COOH^- , $\text{C}_6\text{H}_9\text{O}_2^-$ and $\text{C}_6\text{H}_{11}\text{O}_3^-$, respectively. The stage was translated in 1 mm increments and the signals are plotted to show increasing hydrophilicity.

The trend plots of SIMS data confirm that UVO treatment results in a systematic variation in surface chemistry across the substrate. This data correlates with the surface energy (data not shown) and contact angle data both of which show a steady increase in the hydrophilic nature of the surface (increase in surface energy/decrease in contact angle) with increasing UVO exposure. The enhancement observed for the COOH^- , $\text{C}_6\text{H}_9\text{O}_2^-$ and $\text{C}_6\text{H}_{11}\text{O}_3^-$ secondary ion signals is expected because photo-oxidation is known to increase the polar surface interactions of atoms [2,3]. In the UVO treatment of polymers, the atomic oxygen is thought to react with the carbon chain through insertion or hydrogen-abstraction reactions to produce oxidized entities such as carbonyl and carboxyl groups [4,5]. On this surface the photo-oxidation process results in cleavage of the most labile ester linkage, causing an increase in the surface density of the COOH end functional group. For this same reason, chain fragmentation also enhances the signals for the peaks at m/z 113 and 131 as seen in Fig. 2B and C.

The effect of surface treatment on the bioreceptivity of PCL was then assayed by *in vitro* culture of osteoblast-like mouse calvarial MC3T3 cells. The systematic variation of surface energy over macroscopic distances (≈ 5 cm) provides an ideal test substrate to study the effect of surface energy on cellular reception where all other conditions (soluble factors,

seeding density, film preparation) are identical. The macroscopic distances and relatively low motility cell line were chosen to preclude effects such as chemotaxis. The extent of cell adhesion and cell spreading was found to correlate directly to the contact angle of the substrate. After 72 h, the population of adherent cells on a similarly prepared (cast from dioxane instead of toluene) gradient surface energy substrate decrease monotonically from 200 to 25 mm^{-2} from the relatively hydrophilic ($\theta_{\text{H}_2\text{O}} \approx 40^\circ$) to the hydrophobic end ($\theta_{\text{H}_2\text{O}} \approx 70^\circ$) [6]. The cell adhesion and proliferation were monitored in time. A concomitant four-fold decrease in extent of cell spreading followed the decrease in cell population from the hydrophilic to the hydrophobic region. In a future work, we intend to extend the use of these test substrates and TOF-SIMS to probe the mediating absorbed serum protein distribution that constitutes the biointerface in cellular reception of polymeric materials.

4. Conclusions

TOF-SIMS has allowed us to correlate contact angle measurements with the chemical nature of UVO-treated PCL surfaces. We have demonstrated that UVO treatment results in an increase in COOH end functional group. This increase resulted in increases in the hydrophilicity and biocompatibility of the surfaces. The creation of functionalized surfaces such as these could potentially prove useful in combinatorial studies, as they provide a means of creating multi-parameter spaces on which to conduct assays.

References

- [1] S.V. Roberson, A. Sehgal, A.J. Fahey, A. Karim, *Appl. Surf. Sci.* (2001), submitted for publication.
- [2] M. Ouyang, C. Yuan, R.J. Muisener, A. Boulares, J.T. Koberstein, *Chem. Mater.* 12 (2000) 1591–1596.
- [3] H.-Y. Nie, M.J. Alzak, B. Berno, N.S. McIntyre, *Appl. Surf. Sci.* 144–145 (1999) 627–632.
- [4] D.O.H. Teare, C. Ton-That, R.H. Bradley, *Surf. Interf. Anal.* 29 (2000) 276–283.
- [5] T. Ye, D. Wynn, R. Dudek, E. Borguet, *Langmuir* 17 (2001) 4497–4500.
- [6] Manuscript, in preparation.