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Model multilayer structures for three-dimensional cell imaging

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Abstract

The prospects for SIMS three-dimensional analysis of biological materials were explored using model multilayer structures. The samples were analyzed in a ToF-SIMS spectrometer equipped with a 20 keV buckminsterfullerene (C_{60}^+) ion source. Molecular depth information was acquired using a C_{60}^+ ion beam to etch through the multilayer structures at specified time intervals. Subsequent to each individual erosion cycle, static SIMS spectra were recorded using a pulsed C_{60}^+ ion probe. Molecular intensities in sequential mass spectra were monitored as a function of primary ion fluence. The resulting depth information was used to characterize C_{60}^+ bombardment of biological materials. Specifically, molecular depth profile studies involving dehydrated dipalmitoyl-phosphatidylcholine (DPPC) organic films indicate that cell membrane lipid materials do not experience significant chemical damage when bombarded with C_{60}^+ ion fluences greater than 10^{15} ions/cm^2. Moreover, depth profile analyses of DPPC–sucrose frozen multilayer structures suggest that biomolecule information can be uncovered after the C_{60}^+ sputter removal of a 20 nm overlayer with no appreciable loss of underlying molecular signal. The experimental results support the potential for three-dimensional molecular mapping of biological materials using cluster SIMS.

Keywords: ToF-SIMS; Polyatomic projectile; C_{60}^+; Molecular depth profiling; 3D imaging

1. Introduction

Molecular depth profiling using time of flight secondary ion mass spectrometry (ToF-SIMS) has recently become a prominent tool in the chemical analysis of multilayer organic systems. The acquisition of depth information using mass spectrometric methods has been facilitated by the employment of cluster primary ion sources. The success of polyatomic particles in SIMS molecular depth profiling has been documented using several organic models [1–3]. These analyses demonstrate that cluster projectiles erode organic material at a rate that prevents the accumulation of significant chemical damage at a sample surface [1–3]. Consequently, surface material can be etched using polyatomic particles to expose an undamaged, molecularly intact interface for subsequent mass spectrometric-depth profile analysis. Moreover, if the mass spectrometric analysis involves the acquisition of chemical-specific SIMS images, a three-dimensional molecular map of the sample can be constructed.

The ability of cluster primary ion sources to acquire molecular information in a three-dimensional manner has potential implications in the field of single cell biological research [4]. Particularly, the capability to acquire chemical-specific images as a function of depth in biological cells would provide valuable insight into neurological activity at the cellular and sub-cellular level [4]. Despite the success with organic models, a molecular depth profile of a preserved biological cell using cluster ion mass spectrometry has yet to be achieved. The plausibility and limitations of this prospective application can be better established through the detailed understanding of polyatomic particle bombardment of biologically relevant molecules. Consequently, the research at hand aims to characterize the buckminsterfullerene (C_{60}^+) ion impact of biomolecules through depth-profile studies involving multilayer biological models. Specifically, dehydrated organic films of the fatty acid dipalmitoyl-phosphatidylcholine (DPPC) are bombarded with C_{60}^+ projectiles to determine the susceptibility of membrane lipid material to the accumulation of chemical damage. Moreover, depth profile analyses involving DPPC–sucrose frozen multilayered structures are explored to describe primary cluster ion impact of biomolecules in their preserved native environment of water–ice.

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2. Experimental

The experiments were performed in a ToF-SIMS apparatus equipped with cryogenic cooling capabilities described in detail elsewhere [5]. A 20 keV C_{60}^+ ion source is mounted onto the instrument at a 40° angle with respect to the surface normal [6]. Under typical operating conditions, the fullerene source delivers a 0.5 nA ion current at a 20 μm probe size onto the analysis stage. Computer software was used to automate the instrumental hardware for precise control during molecular depth profiling analysis. More specifically, depth profiling experiments were performed by alternating between sputter erosion cycles and data acquisition cycles. Sputter cycles were achieved by rastering the C_{60}^+ ion beam across the sample surface in direct current mode at a specific field of view and for a specific time. Sputter area and sputter time were selected to deliver a desired primary ion fluence per sputter cycle. Data acquisition cycles were acquired by rastering the C_{60}^+ ion beam in pulsed mode across the sample surface. To eliminate crater effects, the analysis field of view was chosen to be smaller then the etched area. Positive static SIMS spectra were recorded in single ion counting mode and summed over 10^5 ion pulses. To characterize the depth profile, analyte molecular intensities from sequential mass spectra are plotted as a function of primary ion fluence. No charge compensation was required during either the erosion cycles or data acquisition cycles.

The dehydrated DPPC (Avanti Polar Lipids Inc.) film was prepared by spin-casting a lipid chloroform solution (5 mg/mL) onto a chemically etched silicon substrate. The dry, porous organic film was measured to be 1300 nm in thickness and have a 100 nm surface roughness using atomic force microscopy (AFM; Nanopics 2100, TLA Tencor Inc.). Molecular depth profile conditions for the analysis of the DPPC film included a 200 μm × 200 μm sputter field of view for 100 s time intervals and a 100 μm × 100 μm analysis field of view for 50 total cycles. An atomic force micrograph of a C_{60}^+ bombarded DPPC film is shown in Fig. 1a for reference.

The DPPC–sucrose (J.T. Baker Ltd.) frozen multilayer structure was prepared by delivering 5 μL of an aqueous sucrose solution (15 mg/mL) onto a chemically etched silicon substrate. Before the aqueous sucrose solution was allowed to dry, 2 μL of a saturated lipid chloroform solution (15 mg/mL) was dropped onto the aqueous sucrose layer. Several seconds were permitted to allow the chloroform to evaporate and the sample was immersed into a liquid nitrogen reservoir. The procedure resulted in a frozen sucrose crystalline ice layer covered by a dry DPPC film approximately 20 nm in thickness. The thickness of the DPPC layer was calculated from an independent study on the sputter yield of fatty acids by 20 keV C_{60}^+ bombardment [3]. The sample was transferred and analyzed in the ToF-SIMS spectrometer under cryogenic conditions. Molecular depth profile experimental parameters for the study of the DPPC–sucrose structure included a 650 μm × 650 μm sputter field of view for 2 s time intervals and a 150 μm × 150 μm analysis field of view for 50 total cycles. A schematic depiction of the DPPC–sucrose model structure is illustrated in Fig. 2a for reference.

3. Results and discussion

An atomic force micrograph and depth profile analysis of a dehydrated DPPC film bombarded with C_{60}^+ is illustrated in Fig. 1a and b, respectively. The profile is an ion intensity versus

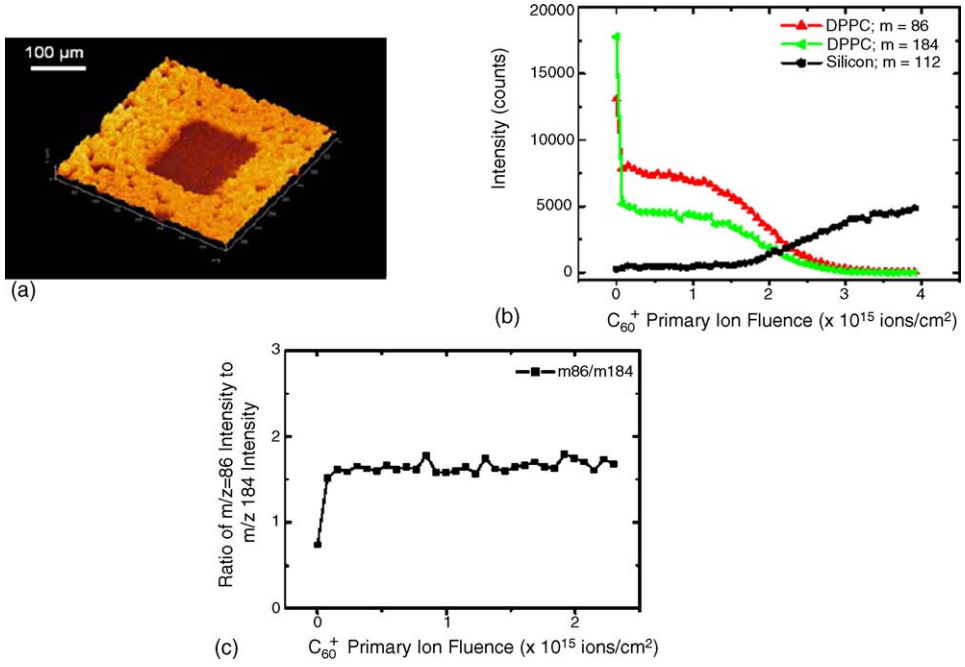

Fig. 1. (a) Atomic force micrograph of a DPPC film bombarded with C_{60}^+ at 200 μm^2 field of view. Image is taken at a 400 μm × 400 μm field of view and indicates the film is 1300 nm thick with a 100 nm surface roughness; (b) depth profile plot of DPPC molecular fragments $C_5H_{15}NPO_4^+$ (m/z = 184), $C_5H_{12}N^+$ (m/z = 86) and silicon vs. C_{60}^+ ion fluence; and (c) plot of the ratio of the fragmented phosphaditylcholine m/z = 86 and the phosphaditylcholine parent ion m/z = 184 as a function of C_{60}^+ ion fluence.

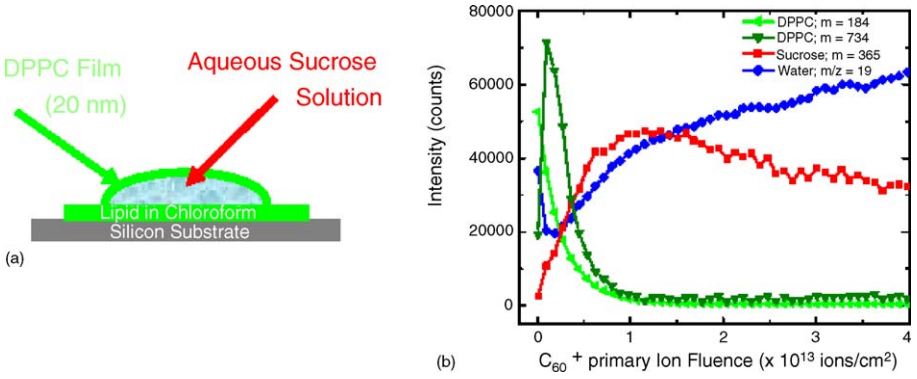

Fig. 2. (a) Schematic representation of DPPC–sucrose frozen multilayer structure, illustration not drawn to scale.; (b) Depth profile plot of DPPC molecular fragment $C_5H_{15}NPO_4^+$ (m/z = 184), DPPC molecule $C_{40}H_{81}NPO_8^+$ (m/z = 734), sucrose–sodium adduct $C_{12}H_{22}O_{11}Na^+$ (m/z = 365), and hydronium water H_3O^+ (m/z = 19) vs. C_{60}^+ ion fluence.

primary ion fluence plot of phosphaditylcholine (m/z = 184; $C_5H_{15}NPO_4^+$), the head group of the DPPC phospholipid, choline (m/z = 86; $C_5H_{12}N^+$), a fragment of the phosphaditylcholine head group, and a silicon cluster (Si_4^+; m/z = 112). DPPC (m/z = 734; $C_{40}H_{81}NPO_8^+$) molecular ion intensity is not readily observed in ToF-SIMS spectra of dehydrated DPPC films [7]. Accordingly, phosphaditylcholine and choline ion intensities are used as a measure of DPPC chemical damage during the C_{60}^+ profile. Specifically, the depth profile of the DPPC film is characterized by three distinct regions: an initial period of chemical damage, an extended quasi-stable sputter-state, and the complete disappearance of DPPC signal at the silicon interface. The broad DPPC film/silicon interface region observed in the profile arises almost entirely from the original 100 nm surface roughness of the film as determined by the AFM measurement. Most notably, the depth profile indicates the DPPC molecule can be sputtered in a controlled manner for C_{60}^+ doses greater than 10^{15} ions/cm^2, a value limited by the bulk thickness of the film. This observation is supported by the measure of molecular fragmentation of the DPPC molecule under C_{60}^+ bombardment throughout the bulk region of the film shown in Fig. 1c. Chemical damage is illustrated in the figure by plotting the ratio of the fragmented phosphaditylcholine ion m/z = 86 and the phosphaditylcholine parent ion m/z = 184 as a function of C_{60}^+ ion fluence. The data indicates that the amount of fragmented ions initially increases slightly with ion fluence but rapidly reaches a steady-state value. This observation suggests that equilibrium is reached between ion beam-induced molecular fragmentation and sputter removal of previously damaged molecules and that molecule-specific information can be retained after repeated C_{60}^+ bombardment. The controlled erosion of the film can be attributed to the large overall sputter yields and shallow penetration depths experienced with C_{60}^+ particle impact [1–3,8]. Similar depth profiles have been achieved using other organic models [1–3]. However, the DPPC model is of specific biological significance since the film consists of a fatty acid lipid molecule that is a major constituent of cell plasma membranes and crucial to cellular function [3]. Thus, the ability to profile through over 1000 nm of the DPPC molecule using C_{60}^+ while retaining molecular signal has major implications in the 3D analysis of single biological cells.

A typical depth profile study of the DPPC–sucrose frozen hydrated multilayer structure (shown in Fig. 2a) using a C_{60}^+ ion beam is illustrated in Fig. 2b. The profile is a plot of phosphaditylcholine ion, DPPC molecular ion, sucrose–sodium adduct (m/z = 365; $C_{12}H_{22}O_{11}Na^+$) ion, and hydronium water (m/z = 19; H_3O^+) ion intensities as a function of C_{60}^+ ion fluence. The most important observation to be made concerning the depth profile of the DPPC–sucrose model is the abrupt disappearance of the overlying DPPC layer and the corresponding appearance of the underlying sucrose at the multilayer interface. Although specific details regarding the formation of topography and the occurrence of interlayer mixing in the profile are not known, the data indicates that lipid films 20 nm in thickness can be etched using C_{60}^+ projectiles to uncover molecular signal from an underlying biological analyte. A second point to be made about the DPPC–sucrose profile involves the initial increase of DDPC molecular ion m/z = 734 intensity and the corresponding decrease in hydronium ion m/z = 19 intensity at the vacuum–lipid film interface illustrated in Fig. 2b. This observation indicates the C_{60}^+ removal of a thin ice overlayer and the consequent uncovering of the DPPC layer. The thin ice overlayer formed as a result of water vapor condensation onto the frozen structure during sample transfer and is calculated to be 0.5 nm in thickness [8]. This data supports the earlier position that C_{60}^+ etching can expose an underlying biological analyte without the incidence of chemical damage. The reason for phosphaditylcholine ion m/z = 184 not experiencing a similar increase in ion intensity as the DPPC molecular ion m/z = 734 is not currently known; however, a possible reason may be the increased tendency of protonation to occur with heavier, slower moving molecules. As a note to complement the DPPC–sucrose profiles, C_{60}^+ molecular depth profile studies involving an ice–histamine frozen multilayer structure demonstrated similar experimental trends [9].

To date, a molecular depth profile of a frozen–hydrated biological cell has yet to be achieved. Thus, the DPPC–sucrose multilayer structure was chosen to model a biological cell in 3D imaging experiments. Specifically, the structure entails a lipid film similar to a plasma cell membrane and an underlying aqueous sucrose solution similar to a cell cytoplasm. Additionally, the structure is frozen in water–ice, the preserved native environment of biological cells. Consequently, the depth

profile data obtained using the DPPC–sucrose model can be employed to comment on the plausibility of 3D molecular mapping of biological cells. Interestingly, the profile results suggest C_{60}^+ 3D analysis of cellular material is possible and the resulting analysis would be characterized by the ability to uncover undamaged biomolecule information from intercellular material with adequate depth resolution and sensitivities.

4. Conclusions

The general objective of this research was to characterize the C_{60}^+ ion impact of biological materials. Accordingly, depth-profile studies involving multilayer biological models were employed to identify several significant properties of C_{60}^+ bombardment. Specifically, C_{60}^+ erosion of biologically important molecules such as membrane lipids was determined not to inflict significant chemical damage to the sample surface. Additionally, C_{60}^+ projectiles were demonstrated to remove overlying lipid films and uncover underlying biomolecules with no appreciable loss of molecular information throughout the profile. These described sputtering characteristics of C_{60}^+ improve the prospects for 3D molecular analysis of single biological cells using cluster SIMS.

Acknowledgements

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