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Depth profiling of taxol-loaded poly(styrene-b-isobutylene-b-styrene) using Ga$^+$ and C$_{60}^+$ ion beams

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Abstract

The surface of a triblock copolymer, containing a solid-phase drug, was investigated using 15 keV Ga$^+$ and 20 keV C$_{60}^+$ ion beams. Overall, the results illustrate the successful use of a cluster ion beam for greatly enhancing the molecular ion and high-mass fragment ion intensities from the surface and bulk of the polymer system. The use of C$_{60}^+$ also established the ability to see through common overlayers like poly(dimethyl siloxane) which was not possible using atomic ion sources. Moreover, the use of C$_{60}^+$ allowed depth profiles to be obtained using primary ion dose densities in excess of 6×10^{14} C$_{60}^+$/cm^2. Resulting sputter craters possess relatively flat bottoms without the need for sample rotation and reached depths of ca. 2 μm. AFM results illustrate the more gentile removal of surface species using cluster ions. Specifically, phase contrast and topographic images suggest the relatively high ion doses do not significantly alter the phase distribution or surface topography of the polymer. However, a slight increase in rms roughness was noticed.
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1. Introduction

In recent years, the use of polymeric biomaterials for in vivo applications has increased dramatically. Hydrogels and derivatives of poly(lactic acid), poly(glycolic acid) and poly(ethylene glycol) have come to center stage in the development arenas with many applications focusing on drug delivery [1]. Furthermore, the use of polymeric systems as coupling agents and passivation layers has extended the applicability of current materials. Although the applications of polymer coatings and materials are vast, this manuscript will focus on the characterization of a non-degradable polymer coating associated with implantable stent technology.

Stents are small, tubular scaffolds used in veins, arteries and ducts within the body to open restrictions. Because of their ability to be expanded and retain their shape, metallic stents have recently become ideal mechanical remedies for a variety of complex chemical problems. However, upon implantation the body's defense mechanisms respond by encapsulating the stent via enhanced tissue growth around the metal lattice. Consequently, the use of uncoated stents has proven to be short-lived and has lead to more advanced systems [2].

Although the buildup of red/white blood cells as well as plaque within arteries, for example, can be abated using medications, slowing tissue growth around implanted devices presents a challenge which has recently been overcome by incorporating a drug into a biocompatible polymer that is coated onto the stent. Coatings of this nature allow sustained release of drugs that delay or eliminate tissue growth [3]. Of particular interest are the nature of chemical species present on the surface and as a function of depth on the Taxus^{TM} Express^{TM} Paclitaxel-eluting coronary stent. This system consists of paclitaxel (PTx or Taxol) dispersed within a poly(styrene-b-isobutylene-b-styrene) (SIBS) polymer coated onto an Express^{TM} stainless steel stent. We will show that it is possible to characterize the surface and bulk of this drug/polymer system using a C$_{60}^+$ ion beam. In addition, sputtering artifacts are shown to be minimal and the presence of species like siloxanes do not significantly hinder the detection of molecular ions; a fact that has plagued the use of atomic ion sources (e.g. Ga$^+$).

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2. Experimental

2.1. Materials and film preparation

Initial investigations involved characterizing individual struts on medical grade stents using Ga^+ ion beams. However, sample-handling procedures were deemed a bit cumbersome for developmental work. Consequently, PTx/SIBS formulations were evaluated using cast films. These films helped refine the ion beam characterization process because of the small amounts of polymer available on individual stent struts as well as the limited number of accessible struts after the stents were placed within the mass spectrometer. Film use also reduced the possibility of non-uniform sputtering due to stent/strut tilt angles since it was not always possible to perfectly align the samples orthogonal to the secondary ion flight path. The PTx/SIBS films were cast from 75:25 toluene:THF solution onto clean polyethylene terephthalate sheets using PTx levels of 0.0 and 8.8 wt.%. Crystalline cGMP paclitaxel was used as received from Hauser Inc. Film formation has been previously investigated using transmission and scanning electron microscopies, differential scanning calorimetry, atomic force micrscopy, X-ray diffraction and nuclear magnetic resonance spectroscopy and deemed the same as coatings placed on Taxus[TM] Express[TM] stents [4].

2.2. Instrumentation

2.2.1. Ga ion data

The use and performance of a TRIFT III (Physical Electronics, USA) TOF–SIMS instrument has been described in detail elsewhere and is briefly discussed below [5]. The instrument is equipped with a liquid metal ion source (Ga^+) that was operated using a bunched 15 keV (impact energy 12 keV), 2 nA dc beam. Pulse widths ranging from 18 to 21 ns, a repetition rate of 10 kHz and analysis areas of ca. 125 μm × 125 μm yielded primary ion doses of ca. 5×10^{12} ions/cm^2.

2.2.2. C_{60} ion data

These data were obtained on a BioTof TOF–SIMS instrument that has been described in detail elsewhere and is briefly discussed below [6]. The instrument incorporates a C_{60}^+ primary ion source from Ionoptika Ltd (Southampton, U.K.), which is directed at a 40° angle to the surface normal. For depth profiling, the C_{60}^+ ion beam was operated at 20 keV in dc mode using 300–600 μm × μm areas followed by the acquisition of TOF mass spectra after each sputter cycle using C_{60}^+ at an ion fluence of 10^{10} cm^{-2}.

2.2.3. AFM results

Samples were tested using a Multimode AFM (Digital Instruments/Veeco Metrology) controlled with Nanoscope IIIA, Extender electronics. The cantilever was a Tap300 Metrology Probe (Nanodevices, Santa Barbara, CA) with the following parameters: single-arm silicon; length = 120 μm; spring constant = 40 N/m; resonant frequency = 300 kHz; probe tip ~10 nm radius of curvature. For tapping mode

measurements, the piezo drive amplitude produced a 4 V near-surface, non-contact cantilever oscillation signal and the contact attenuation set-point was fixed at 3 V.

2.2.4. AFP

Crater depths, edge profiles and calculated sputter rates were determined using a KLA-Tencor Nanopics2100 atomic force profilometer which provides 800 μm scan ranges. Resolution in the Z and X/Y ranges are ca. 3 Å and 2 nm, respectively. Tips used during experiments have the following characteristics: single-arm silicon; spring constant = 4 N/m; radius = 20 nm; conical shape (20° initial angle, 60° at flare point). The contact-mode force exerted by the tip was 1150 nN.

3. Results and discussion

Initial characterization of the PTx-loaded SIBS polymer was performed using a Ga^+ ion source. As shown in Fig. 1, the $(M + H)^+$ ion from taxol is not observed. Moreover, the presence of polydimethyl siloxane (PDMS), which is innate to the polymer formulation, made routine characterizations with Ga^+ ions challenging since the material often migrated across the sample surface. Consequently, a method to enhance the detection sensitivity of higher mass species as well as a way to see 'through' the PDMS was sought. After considering initial work [7] as well as more recent investigations using cluster ion sources [8], it became apparent that it may be possible to
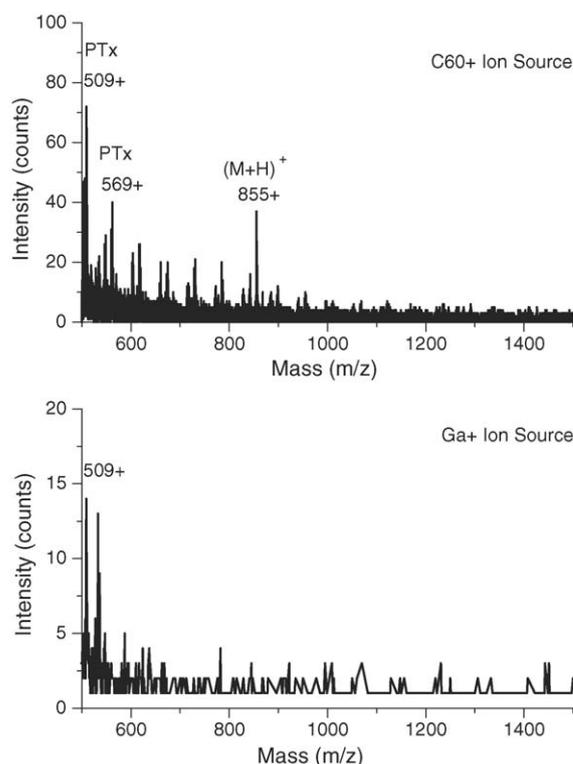

Fig. 1. Positive, secondary ion mass spectra of SIBS polymer containing PTx. Data were recorded using Ga^+ ions (lower spectrum) and C_{60}^+ ions (upper spectrum) under static conditions (i.e. primary ion doses below 1×10^{12} ions/cm^2). The spectra clearly show detection of higher mass species using C_{60}^+ primary ions.
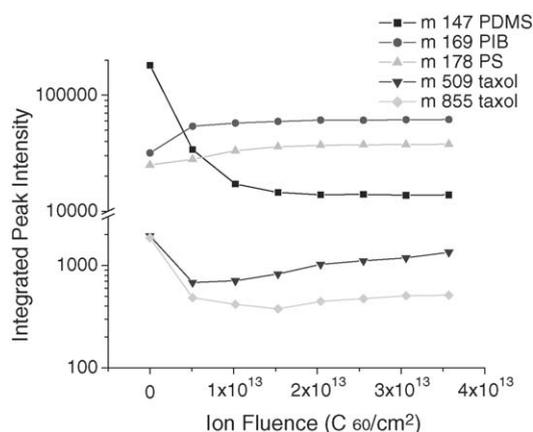
Fig. 2. Depth profile of SIBS polymer containing PTx. Secondary ion intensities for select species plotted as a function of primary ion (C_{60}^+) dose.

minimize the contribution of contamination layers like PDMS and extract information that was more chemically relevant to the material in question. Interestingly, relatively few systems had been investigated using C_{60}^+ ions at the time of consideration, but they showed great promise in changing the way chemical information is extracted from the surface and bulk regions of polymers [9,10]. Fig. 1 also shows the relative enhancements associated with the molecular ion of PTx and higher-mass fragments using C_{60}^+ primary ions. Previous attempts to detect the molecular ion of PTx using Ga^+ were unsuccessful even though the molecular ion was observed in spectra from reference materials.

Based on the increase in higher-mass secondary ion peak intensities using C_{60}^+ on PTx/SIBS and previous successes noted above, we postulated that it may be possible to obtain a three dimensional distribution of species within this biomaterial. Fig. 2 shows the intensity of secondary ion species from the
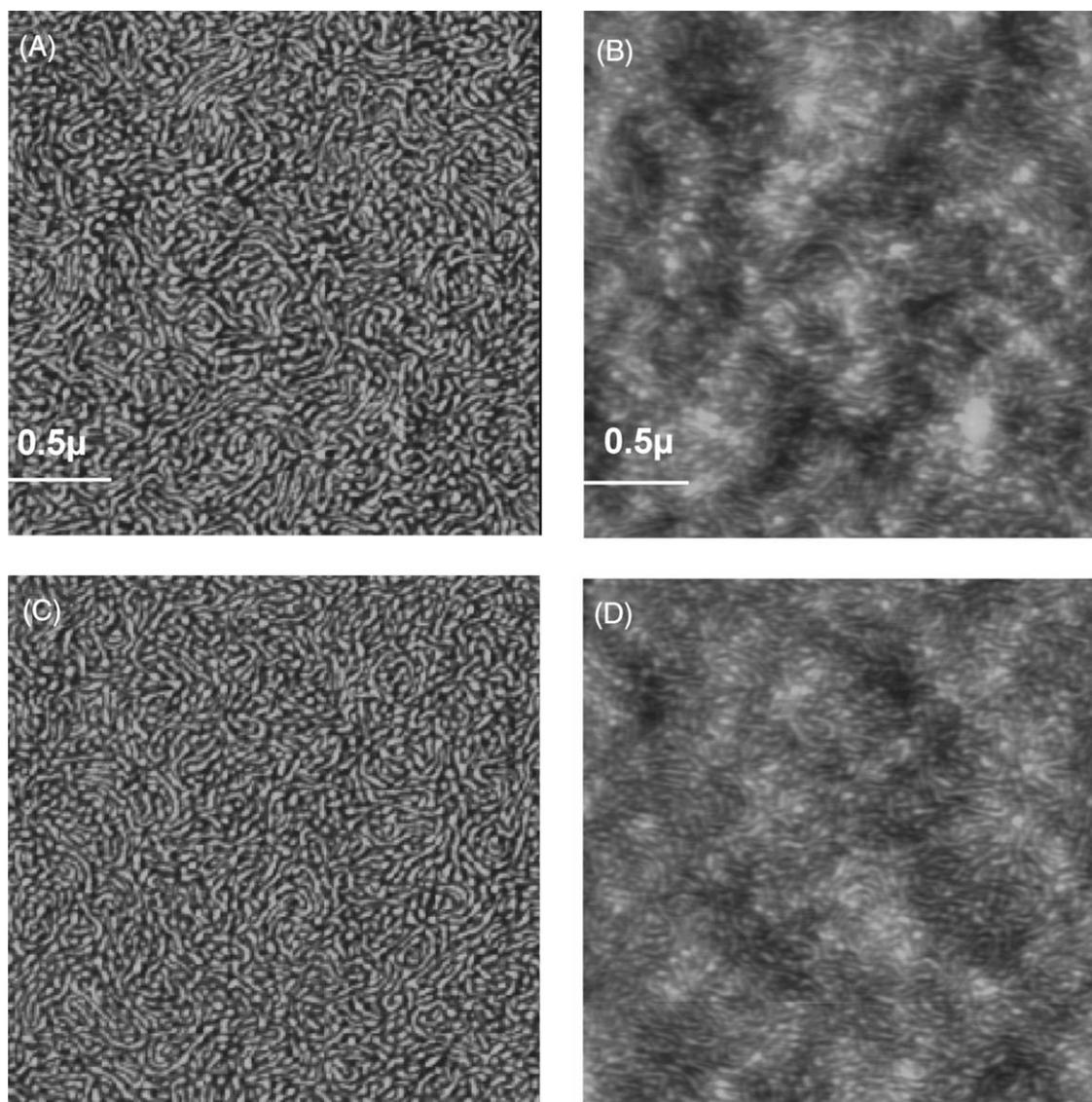

Fig. 3. Atomic force microscopy images acquired from 2.5 μm × 2.5 μm areas before and after sputtering using a primary ion dose of 4×10^{14} C_{60}^+/cm^2. Top images show phases contrast (A) and topography (B) images from the same area within the sputter crater. Images (C) and (D) are obtained from a control region outside the crater. Light areas correspond to polystyrene and darker regions correspond to polyisobutylene.

polymer matrix plotted as a function of primary ion dose (C_{60}^+/cm^2). Peaks related to poly(styrene) and poly(isobutylene) fragments possess slightly lower intensity in the surface region and achieve a relatively uniform intensity profile (i.e. steady state) at higher ion doses. In contrast, the PDMS and taxol related peaks possess higher intensity near the surface and approach a steady-state intensity after a primary ion dose of ca. 2×10^{13} ions/cm^2. If a so called matrix effect were to alter ion yields as a function of sputter time then all species should be affected and show similar intensity distributions [11]. Consequently, it is reasonable to assess that there is an enrichment of PDMS and PTx at the surface of the SIBS polymer. However, it is not possible to quantitate these findings since the sputter and ion yields as well as disappearance cross-sections for the individual components are unknown. It is worth noting that all characteristic fragment ions from the above moieties showed the same intensity distributions versus primary ion dose.

With sputter yields and disappearance cross-sections in mind, one may consider the relative damage primary ions induce during sputtering. Information about the three dimensional distribution of species would be limited if the sample were significantly damaged during the bombardment process. One indicator of damage is the alteration of surface topography. Consequently, atomic force microscopy was utilized to glean information about the polymer components as well as for the assessment of surface roughness. Fig. 3 shows examples of phase contrast images obtained before and after sputtering. The phase of the oscillating AFM tip clearly defines the copolymer domains [12], which are consistent with the non-sputtered surface even after primary ion doses of 7×10^{14} C_{60}^+ ions/cm^2. In comparison, constant height images indicate the rms roughness values for the copolymer are slightly higher (ca. 10 nm) after sputtering.

After establishing the size and depth (ca. 2 μm) of the sputter craters using a stylus profilometer it was possible to determine that the cavity had a relatively flat bottom. In fact, all craters and profiling studies were produced without the need of sample rotation. From the crater measurements, we were able to calculate approximate sputter rates associated with the use of C_{60}^+. For the range of craters used in the above studies, calculated sputter rates varied from 1.9 to 19 nm/s depending on the ion beam current and raster size. To minimize possible artifacts associated with edge regions the width of the rastered area for the sputter probe was ca. three times larger than the width of the rastered analysis probe. Clearly, the combination of low beam damage, high mass sensitivity, flat-bottom profiles (i.e. good depth resolution) and the ability to quickly remove material has further established the use of C_{60}^+ ion sources for characterizing complex polymer systems and may well extend to the observation of biological activity within cellular systems in future studies.

4. Conclusions

We have taken advantage of the enhanced sensitivity and ability for molecular depth profiling of cluster ion beams to characterize a drug-containing polymer. The C_{60}^+ source showed a noticeable improvement in removing and/or minimizing the effects of overlayers (i.e. polydimethyl siloxane) that have previously stifled acquisitions using Ga$^+$ ion beams. With respect to depth profiling and the three dimensional distribution of species, we conclude that the surface of the polymer film is slightly enriched with Taxol and PDMS. After primary ion doses of approximately 2×10^{13} C_{60}^+ ions/cm^2, steady state signals were observed and sustained through ion doses exceeding 6×10^{15} ions/cm^2. Interestingly, the use of C_{60}^+ to characterize this system does not appear to significantly alter the domain structure of the copolymer as confirmed by topography and phase contrast AFM images. Based on these findings we feel the use of C_{60}^+ ion beams will greatly enhance the characterization of more complex polymers as well as ex vivo samples where the potential for surface contamination and bio-fouling is greatest. These types of investigations would be severely hindered using Ga$^+$ ion sources.

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