

3D chemical characterization of frozen hydrated hydrogels using ToF-SIMS with argon cluster sputter depth profiling

Michael Taylor, David Scurr, Matthias Lutolf, Lee Buttery, Mischa Zelzer, and Morgan Alexander

Citation: *Biointerphases* **11**, 02A301 (2016); doi: 10.1116/1.4928209

View online: <https://doi.org/10.1116/1.4928209>

View Table of Contents: <https://avs.scitation.org/toc/bip/11/2>

Published by the [American Vacuum Society](#)

ARTICLES YOU MAY BE INTERESTED IN

Multivariate ToF-SIMS image analysis of polymer microarrays and protein adsorption

Biointerphases **10**, 019005 (2015); <https://doi.org/10.1116/1.4906484>

Study of the effect of dipole interactions on hyperthermia heating the cluster composed of superparamagnetic nanoparticles

AIP Advances **5**, 127232 (2015); <https://doi.org/10.1063/1.4939514>

Optically excited nanoscale ultrasonic transducers

The Journal of the Acoustical Society of America **137**, 219 (2015); <https://doi.org/10.1121/1.4904487>

Surface analysis using a new plasma assisted desorption/ionisation source for mass spectrometry in ambient air

Review of Scientific Instruments **83**, 063503 (2012); <https://doi.org/10.1063/1.4729120>

Optical properties of magnetron-sputtered and rolled aluminum

Journal of Applied Physics **98**, 083505 (2005); <https://doi.org/10.1063/1.2085315>

Nanowear in scanning force microscopy: Information on deposits formed in and downstream of a hexane plasma

Journal of Applied Physics **91**, 3841 (2002); <https://doi.org/10.1063/1.1446220>

Spectra
Simplified

Plot, compare, and validate
your data with just a click

eSpectra:
surface science

SEE HOW IT WORKS



3D chemical characterization of frozen hydrated hydrogels using ToF-SIMS with argon cluster sputter depth profiling

Michael Taylor^{a)} and David Scurr

School of Pharmacy, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom

Matthias Lutolf

Laboratory of Stem Cell Bioengineering, École Polytechnique Fédérale de Lausanne, Lausanne CH-1015, Switzerland

Lee Buttery, Mischa Zelzer, and Morgan Alexander

School of Pharmacy, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom

(Received 8 May 2015; accepted 24 June 2015; published 7 August 2015)

Hydrogels have been used extensively in bioengineering as artificial cell culture supports. Investigation of the interrelationship between cellular response to the hydrogel and its chemistry ideally requires methods that allow characterization without labels and can map species in three-dimensional to follow biomolecules adsorbed to, and absorbed into, the open structure before and during culture. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) has the potential to be utilized for through thickness characterization of hydrogels. The authors have established a simple sample preparation procedure to successfully achieve analysis of frozen hydrated hydrogels using ToF-SIMS without the need for dry glove box entry equipment. They demonstrate this on a poly(2-hydroxyethyl methacrylate) (pHEMA) film where a model protein (lysozyme) is incorporated using two methods to demonstrate how protein distribution can be determined. A comparison of lysozyme incorporation is made between the situation where the protein is present in a polymer dip coating solution and where lysozyme is in an aqueous medium in which the film is incubated. It is shown that protonated water clusters $H(H_2O)_n^+$ where $n = 5-11$ that are indicative of ice are detected through the entire thickness of the pHEMA. The lysozyme distribution through the pHEMA hydrogel films can be determined using the intensity of a characteristic amino acid secondary ion fragment. © 2016 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution 3.0 Unported License. [<http://dx.doi.org/10.1116/1.4928209>]

I. INTRODUCTION

Three-dimensional polymer networks that hold large quantities of water are termed hydrogels.¹ This class of materials has been investigated extensively in biomedical research over the past decade due to their attractive properties and biophysical similarities to soft biological tissues.² Certain synthetic hydrogels have been proposed to be similar in composition and structure to the native extracellular matrix of the stem cell niche, their *in vivo* cell habitat, which is a powerful component in controlling stem cell fate.³ Ease of chemical modification, biocompatibility, gas permeability, and the ability to deliver functional compounds are some of the attractive properties that may be exploited to instruct stem cell development on hydrogel substrates.⁴⁻⁷ In many hydrogel culture substrates, bioactive compounds are incorporated to illicit control of stem cell regulatory mechanisms. The choice of cell developmental pathway taken can be strongly dependent on the 3D hydrogel chemistry, which plays a significant role in determining hydrogel–cell interactions⁸ in addition to their physical properties.⁹⁻¹¹ Promotion of osteogenesis, adipogenesis, and chondrogenesis of human mesenchymal stem cells on poly(ethylene glycol) (PEG)

based hydrogels has been described through simple functional group modifications to hydrogel chemistry.⁸

Stem cells cultured upon or within biologically derived hydrogels such as MatrigelTM actively remodel the hydrogel, exuding extracellular proteins and soluble factors that create a favorable niche environment in which to reside.^{12,13} Synthetic hydrogel chemistry can be designed to respond to remodeling. PEG hydrogels have been reported where severable matrix metalloproteinase (MMP) responsive peptide linkages are incorporated into the polymer backbone, allowing active remodeling by MMP hydrolysis of the responsive peptide fragments.¹⁴ This illustrates the characterization challenge to gaining a complete understanding of the chemical processes involved in stem cell culture on hydrogels.

Previous work in surface characterization has shown time-of-flight secondary ion mass spectrometry (ToF-SIMS) to be a powerful tool in material characterization, particularly in understanding the complex surface chemistry of extracellular matrices.¹³ Subsurface chemical characterization of such organic systems is now possible using polyatomic sputtering beams such as Ar cluster ions beams in combination with a focused liquid metal ion analysis source. Ejected secondary ions from the materials are analyzed as a function of etch time creating a depth profile of the material.¹⁵⁻¹⁷ Bailey *et al.* recently demonstrated the Ar cluster source's capacity to reliably sputter through multilayer

^{a)} Author to whom correspondence should be addressed; electronic mail: pazma1@exmail.nottingham.ac.uk

polymer films of varying thickness ($\leq 15 \mu\text{m}$).¹⁸ Through thickness chemical characterization of hydrogels will ideally be able to minimize changes associated with dehydration or the increase complexity of chemical fixation.¹⁹

The preparatory of a frozen hydrated sample is not trivial; it must be suitable to both retain water in the vacuum environment and limit ice formation on the sample surface.^{20–25} We describe a preparation procedure for introducing hydrogels into a ToF-SIMS instrument for frozen hydrated 3D chemical analysis that employs a gas to remove crystals of frozen, condensed water from the sample surface prior to evacuation of the instrument load lock. In this study, we compare the results of cryodepth profiling of a simple polymer hydrogel system of poly(2-hydroxyethyl methacrylate) (pHEMA) with a model protein (lysozyme) either premixed with the polymer or immersed in a solution to allow it to diffuse into the hydrogel film. This simple method of preparing pHEMA hydrogel films and introducing them into the ultrahigh vacuum instrument provides high quality ToF-SIMS depth profiles.

II. EXPERIMENT

A. Materials

Poly(2-hydroxyethylmethacrylate) (6% wt/v, $M_n \sim 20\,000$, Sigma Aldrich) was dissolved in ethanol by sonication overnight at 20°C . A silicon wafer substrate ($5 \times 5 \text{ mm}$) was prepared by first sonicating with water, acetone, and finally with ethanol. The wafer was dip coated in the pHEMA solution at a retraction speed of 2 mm/s three times and allowed to dry for 1 h. To investigate the ingress of protein into the films, the polymer dip coated wafer was immersed in a lysozyme (1% wt/v, Sigma Aldrich)/Milli-Q grade deionized water solution at room temperature for 16 h. A pHEMA (6% wt/v) and lysozyme (1% wt/v) ethanol solution was also prepared and dip coated onto silicon wafers. Similarly, the silicon wafers was immersed in deionized water for 16 h. Ellipsometric measurements of the films before analysis indicated an average dry film thickness of 300 nm for the hydrated pHEMA hydrogel in the absence of lysozyme. Measurement of film thickness of the pHEMA/lysozyme hydrogels was unavailable due to the opaque nature of the films.

B. Frozen hydrated sample preparation

For ToF-SIMS analysis, hydrated films were mounted onto an ION TOF cryostage, one sample at a time (*of dimensions* $5 \times 5 \text{ mm}$) followed by plunging into liquid nitrogen for 5 min. The sample stage was removed from the liquid nitrogen and placed onto the precooled sample transfer arm in the entry chamber under nitrogen flow ($T \sim -70^\circ\text{C}$). A high flow of argon gas (3 bars) was immediately used to remove ice (frost), which formed on the sample stage in an “air gun” fashion, which accumulated upon transfer through the ambient air to the loadlock. Upon reaching a pressure of $2 \times 10^{-6} \text{ mbar}$, the entry door to the main chamber was opened and the cryostage introduced for analysis. Sample temperature did not exceed -70°C in the entry chamber, maintaining at -110°C under a $2 \times 10^{-6} \text{ mbar}$ vacuum. Analysis was performed at -120°C in the main chamber; the sample was

maintained below this temperature when depth profiled to reduce the possibility of surface ice crystallization.

C. Instrumentation

ToF-SIMS data were collected using a ToF-SIMS IV instrument (Münster, Germany) equipped with a bismuth liquid metal ion gun and argon cluster sputter gun. The analysis beam for this study was generated by the liquid metal ion gun, specifically a 25 keV bismuth source, utilizing a Bi_3^{++} rastered over an area of $100 \times 100 \mu\text{m}$ with 128×128 pixels. The target current was measured as 0.3 pA with a total primary ion dose of $9.2 \times 10^{10} \text{ ions/cm}^2$. A 10 keV Ar_{1455} cluster ion source was employed to etch through the sample over a $400 \times 400 \mu\text{m}$ area. Rastering was performed in a noninterlaced mode with 1 frame of analysis and 3 s for sputtering per cycle. The corresponding beam dose was determined to be $6.84 \times 10^{12} \text{ ions/cm}^2$. An argon beam target current of 9 nA was employed for all samples analyzed allowing comparison of sample thicknesses from time taken to reach the hydrogel–silicon wafer interface. Postprofiling crater analysis was attempted with the aim of determining the crater depth for each sample, however, on warming the films to room temperature topography developed, likely indicating delamination of the films, preventing accurate film thickness analysis. A low energy electron flood gun was employed for charge neutralization. Data processing was done with the commercial ION-TOF software, SURFACELAB6. Secondary ion assignments were selected by referring to a reference database of secondary ions related to the components of the material analyzed.²⁶

III. RESULTS

The process of analyzing frozen hydrated pHEMA samples by ToF-SIMS involves plunge freezing in liquid nitrogen, which then leads to the accumulation of surface frost during the short transfer in the ambient laboratory atmosphere. Etching of these samples provides very poor depth profiles since the spectra are taken from the rough surface of ice accumulated on the flat sample. While dry box entry is a solution to this problem, we investigated the simpler approach of removing the frost from the surface of the sample using pressurized gas and immediately evacuating the entry lock.

Three frozen hydrated samples are compared in this study, including a pHEMA film, a pHEMA film immersed in lysozyme solution, and lysozyme mixed with the pHEMA dip solution used to form a film. The positive polarity secondary ion spectra acquired using Bi_3^{++} from the surface of the samples are presented in Fig. 1. All the spectra are dominated by secondary ion peaks characteristic of pHEMA, such as at $m/z = 39, 41, 43, 45$, and 69 (labeled with red markers in Fig. 1), the corresponding formulas for which are shown in Table I. Protonated water clusters at $m/z = 91, 109, 127, 145$, and 199 representative of $\text{H}(\text{H}_2\text{O})_5^+$, $\text{H}(\text{H}_2\text{O})_6^+$, $\text{H}(\text{H}_2\text{O})_7^+$, $\text{H}(\text{H}_2\text{O})_8^+$, and $\text{H}(\text{H}_2\text{O})_{11}^+$, respectively, were additionally observed (highlighted with blue markers in Fig. 1). Histidine is also indicated as a marker for lysozyme by the $\text{C}_4\text{H}_6\text{N}_2^+$ peak at $m/z = 82$ (green marker in Fig. 1).²⁶ A

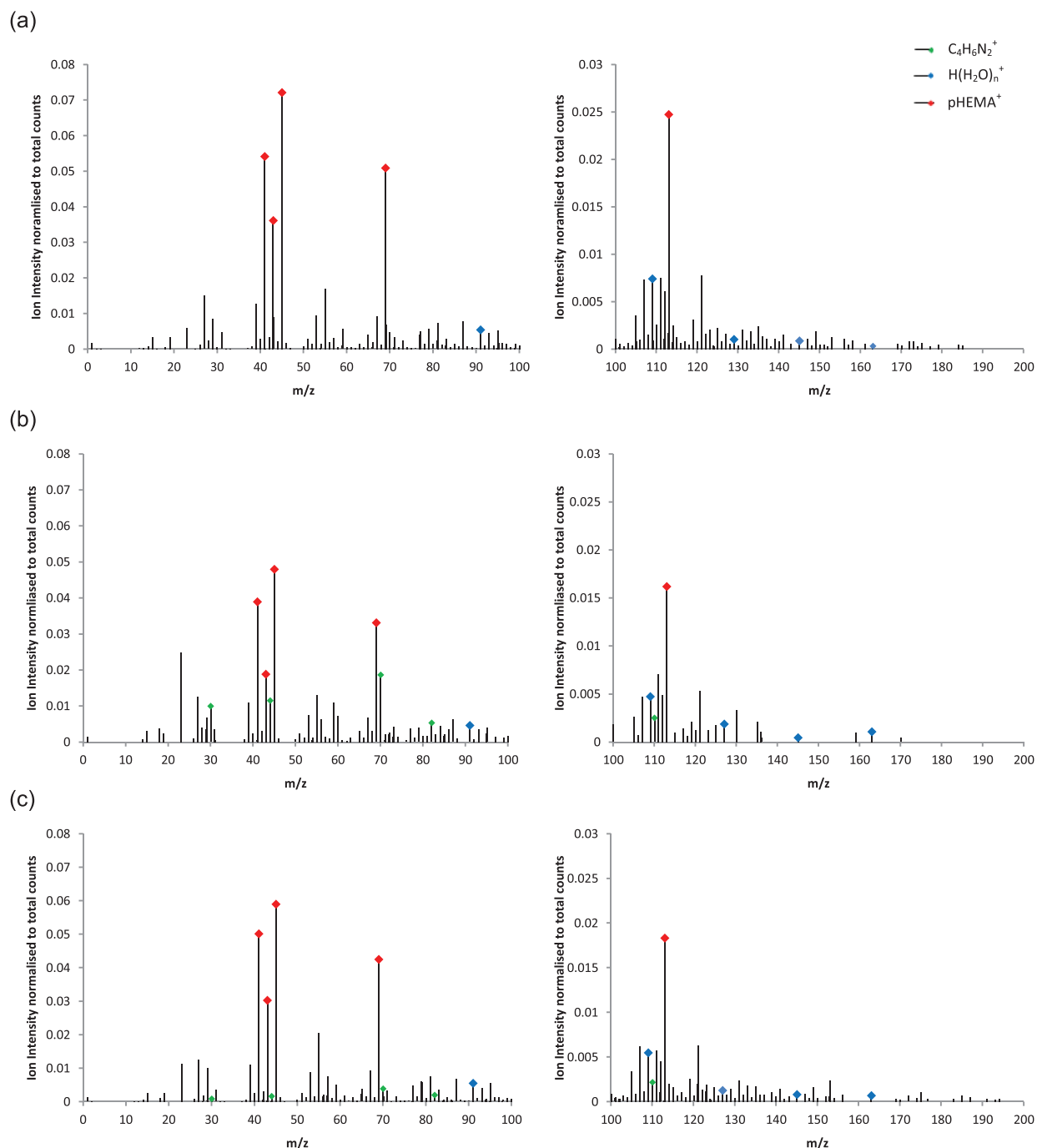


FIG. 1. Positive polarity ToF-SIMS spectra acquired from five scans of the hydrogel surface followed by incubating in water for 16 h at 20 °C for (a) a pHEMA film, (b) a pHEMA hydrogel incubated in 1% wt/v lysozyme in water for 16 h at 20 °C, and (c) a silicon wafer dip coated in an ethanoic solution of 6% wt/v pHEMA/1% wt/v lysozyme.

peak at $m/z = 82.182$ was detected in the pure pHEMA sample; however, this was reflective of polymer. The mass peak at $m/z = 82.047$ was absent from this control sample.

A representative secondary ion diagnostic of each component of the sample was selected to represent pHEMA ($C_6H_9O_2^+$), lysozyme ($C_4H_6N_2^+$), protonated water signifying ice ($H(H_2O)_6^+$) and the silicon substrate (Si^+). Depth profiles were observed for the pHEMA film, a pHEMA film incubated in lysozyme solution and a pHEMA/lysozyme film produced by dip coating into a combined lysozyme/

pHEMA coating solution shown in Figs. 2(a), 2(b), and 2(c), respectively. The depth profile of the pHEMA film with water [Fig. 2(a)] suggests a surface enrichment of water and depletion of pHEMA evident in the ion intensity shifts from the first five scans. The intensity of the fragment representing these components then remains constant until the relatively sharp pHEMA-silicon interface observed after 120 s of sputtering. Beyond this point, the spectrum is dominated by the Si^+ peak of the silicon wafer substrate at much lower overall intensity compared to the polymer film.

TABLE I. Positive secondary ions reported from depth profiling pHEMA hydrogels. The shading denotes each component of the hydrogel. Silicon is shown in grey, protein in green, water in blue and pHEMA in red.

	m/z	Structure	Assignment deviation (ppm)		
			pHEMA	(pHEMA/lysozyme)	pHEMA/lysozyme
Silicon wafer	18.038	NH ₄ ⁺	28.2	32.2	40.1
	19.020	H(H ₂ O) ⁺	39.8	35.2	33.5
	22.999	Na ⁺	27.0	83.7	61.1
	27.034	C ₂ H ₃ ⁺	14.8	10.8	13.9
	27.987	Si ⁺	−22.9	−36.4	—
	29.047	C ₂ H ₅ ⁺	2.7	32.2	77.3
	30.023	CH ₄ N	—	12.4	11.9
	39.030	C ₃ H ₃ ⁺	5.7	5.4	20.5
	41.041	C ₃ H ₅ ⁺	26.5	4.3	50.0
	43.022	C ₂ H ₃ O ⁺	28.5	27.6	52.3
	44.218	C ₂ H ₆ N ⁺	—	−2.2	−4.6
	45.040	C ₂ H ₅ O ⁺	39.3	33.0	67.8
	53.043	C ₄ H ₅ ⁺	22.2	18.5	14.4
	55.069	C ₄ H ₇ ⁺	34.3	33.3	35.6
	69.044	C ₄ H ₅ O ⁺	43.4	32.2	68.9
	70.712	C ₄ H ₈ N ⁺	—	10.7	21.1
	82.047	C ₄ H ₆ N ₂ ⁺	—	−61.5	−48.5
	87.051	C ₄ H ₇ O ₂ ⁺	37.4	31.2	59.9
	91.688	H(H ₂ O) ₅ ⁺	−48.2	−56.3	−34.6
Water (n = 6)	109.086	H(H ₂ O) ₆ ⁺	8.7	−2.0	24.0
pHEMA	110.08	C ₅ H ₈ N ₃ ⁺	—	17.4	31.2
	113.089	C ₆ H ₉ O ₂ ⁺	51.2	39.4	73.3
	127.096	H(H ₂ O) ₇ ⁺	4.3	0.2	4.4
	145.113	H(H ₂ O) ₈ ⁺	64.5	52.0	65.8
	182.123	H(H ₂ O) ₁₀ ⁺	−29.3	−36.6	−37.4
	199.130	H(H ₂ O) ₁₁ ⁺	0.9	6.3	−7.8

As can be seen in Fig. 2, the depth profile for the hydrogel film incubated in a lysozyme solution, both the C₆H₉O₂⁺ and H(H₂O)₆⁺ ion intensities significantly increase from the surface compared to the bulk of the film. The C₄H₆N₂⁺ ion, however, displays relatively a steady state ion intensity throughout the profile until reaching the interface between silicon wafer and hydrogel after 110 s of sputtering. A gradual decrease in the C₆H₈O₂⁺ ion intensity is observed, with no steady state being achieved. A decreasing ion intensity was observed over five comparative samples, demonstrating that this effect is repeatable (Fig. 3).

The depth profile for the hydrogel film produced from dip coating a silicon wafer in a pHEMA/Lysozyme dip solution displays a more pronounced increase in the lysozyme secondary ion marker (C₄H₆N₂⁺) at the surface [Fig. 2(c)]. This surface enrichment effect was observed over five samples, suggesting repeatability. The relative intensities of the C₆H₈O₂⁺ and H(H₂O)₆⁺ ions increase from the surface, reaching a steady state intensity after 60 s of sputtering. The relative ion intensity of the C₄H₆N₂⁺ ion at (400 counts) at the surface reduced to a steady state intensity (200 counts) in the bulk of the film.

IV. DISCUSSION

This study aims to develop a simple sample preparation procedure for ToF-SIMS to achieve a frozen hydrated

hydrogel for analysis without dry box sample entry facilities. The method is anticipated to be equally applicable to other vacuum based analytical techniques. We determined that water was retained in all samples by detection of H(H₂O)_n⁺ secondary ions signifying a frozen hydrated state. Previous studies have reported ToF-SIMS molecular depth profiling of ice indicated by the detection of these clusters in a histidine/ice film.^{16,27} The spectra from depth profiling each of the three hydrogel sample compositions show evidence of water cluster secondary ions beginning at n = 5 (m/z = 91.688) to n = 11 (m/z = 199.130), which shows a similarity to the spectra observed previously for pure ice films.¹⁶

Frost build up was removed with a high flow rate of argon to clear the surface of ice crystals, which were accumulated during the transfer between plunging with liquid nitrogen to the precooled sample entry chamber. A time of 4 s argon flow over the sample surface was found to be sufficient to remove surface ice without inducing visible changes in the sample surface. Purging of the entry chamber was also employed with nitrogen to eliminate the condensation of species on the sample surface. Maintaining a frozen sample state between the liquid nitrogen plunge and the analysis chamber via the precooled sample entry chamber was a significant consideration as the sublimation rate of ice in the vacuum chamber increases as sample temperature rises. Sample mounting onto the IonTOF cryostage was time

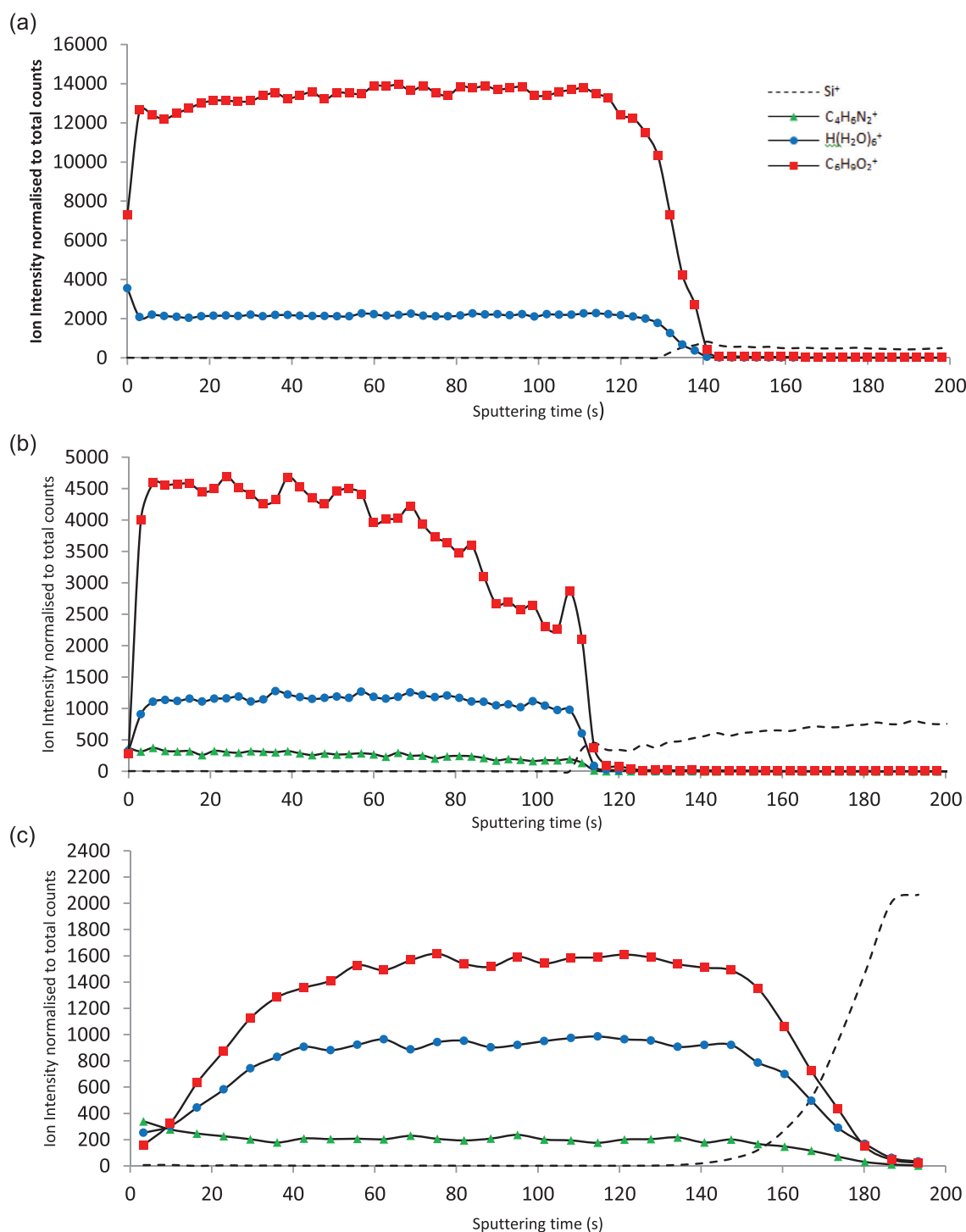


FIG. 2. ToF-SIMS depth profiles of pHEMA hydrogels incubated in water of water/lysozyme for 16 h at 20 °C. (a) A pHEMA film, (b) a pHEMA hydrogel incubating the pHEMA films in 1% wt/v lysozyme in water for 16 h at 20 °C, and (c) a silicon wafer dip coated with a 6% wt/v pHEMA/1% wt/v lysozyme.

consuming; thus, plunge freezing the whole stage assembly allowed immediate transfer of samples to the precooled entry chamber as this did not have any measureable detrimental effect on the instrument vacuum. Previous works by Gemmei-Ide *et al.* among others report a target temperature range of -70 to -110 °C for sample preparation of frozen samples for vacuum analysis, and state that if the sample temperature exceeds -70 °C ice crystallization can occur.^{28,29}

Furthermore, the sample transfer time from liquid nitrogen immersion to the entry chamber was also performed rapidly; otherwise, the accumulation of frost on the sample surface occurs when the sample is exposed to the ambient atmosphere. The entry chamber cold finger was precooled to liquid nitrogen temperature, and upon entry of the precooled sample, the cold finger was immediately connected with the sample stage. The attached thermocouple temperature reading

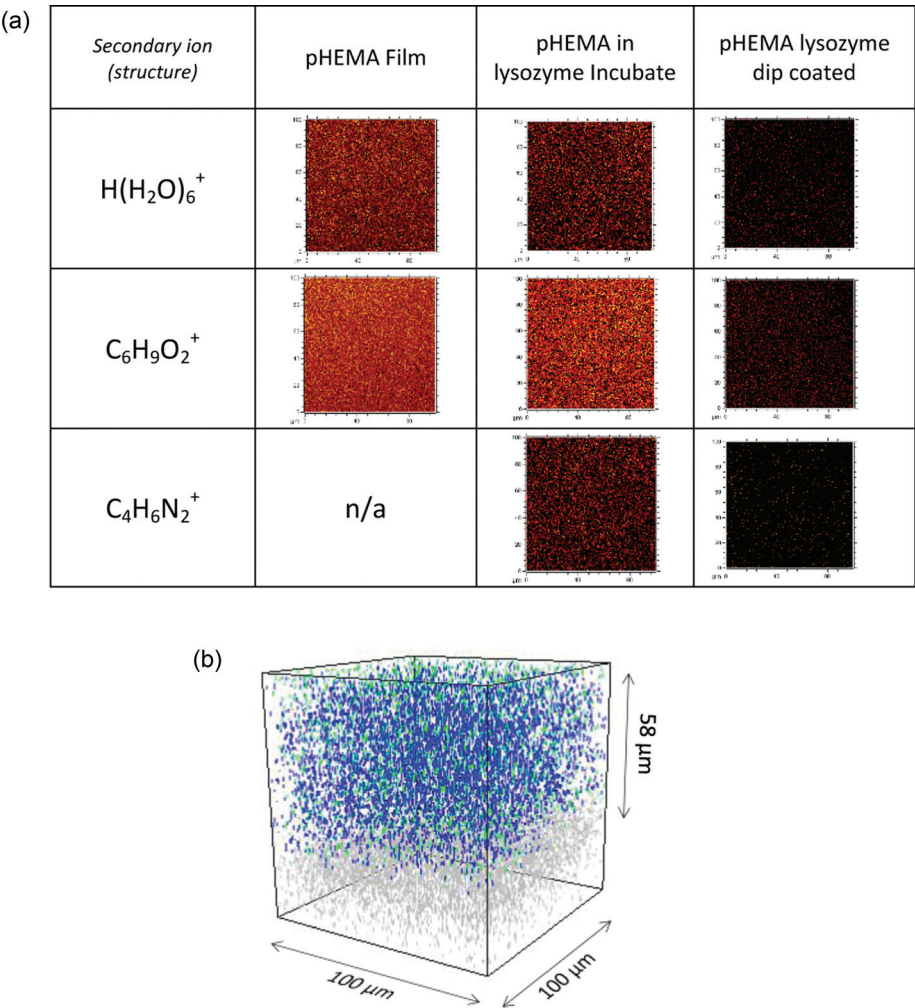


FIG. 3. (a) Positive secondary ion maps from depth profiles of pHEMA hydrogels showing lateral distribution (X/Y coordinates) acquired at 60 s of sputtering. (b) Three-dimensional render of pHEMA/lysozyme film prepared by dip coating in lysozyme/pHEMA solution. $\text{C}_4\text{H}_6\text{N}_2^+$ (Lysozyme) is represented in green, $\text{H}(\text{H}_2\text{O})_6^+$ (ice) in blue, and Si^+ (silicon wafer) in gray. The hydrogel z thickness represents the gel in a dry state, therefore will be an underestimate of the true thickness.

from the cold finger was reflective of the actual sample temperature, therefore could be used to ensure the sample was below -70°C . The following low temperature analysis of each of the sample formats was effective to produce a full through-thickness chemical map. This method has been employed across three differing sample formats showing the versatility of the preparatory procedure in high vacuum frozen hydrated hydrogel analysis.

Additionally, this study aimed to characterize biomolecule distribution within hydrogels using the method developed. A model biomolecule, lysozyme, was used and incorporated within the hydrogel either by diffusion into the pHEMA film during incubation in an aqueous solution or by

mixing with the polymer with the pHEMA solution prior to depositing the film. In both cases, lysozyme was detected throughout the film. Using the relative $\text{C}_4\text{H}_6\text{N}_2^+$ ion intensities within a sample of constant water and polymer content is thought to be valid to characterize the protein distribution as a function of depth, but between samples of composition, the mass spectrometry matrix effect needs to be considered. The most marked difference in secondary ion intensities is the decrease of the intensity of both the characteristic polymer and water upon lysozyme incorporation (Fig. 2). This suggests that the change in chemistry of the sample associated with the introduction of lysozyme suppresses secondary ion emission (Table II) since we know that the molar

TABLE II. Ion intensity ratios at steady state intensity in depth profile, $n = 5$. Standard deviations presented in parentheses.

	$\text{H}(\text{H}_2\text{O})_6^+/\text{C}_6\text{H}_9\text{O}_2^+$	$\text{C}_4\text{H}_6\text{N}_2^+/\text{H}(\text{H}_2\text{O})_6^+$	$\text{C}_4\text{H}_6\text{N}_2^+/\text{C}_6\text{H}_9\text{O}_2^+$
(1) pHEMA	0.18 (0.013)	—	—
(2) Lysozyme incubated pHEMA	0.29 (0.025)	0.32 (0.038)	0.094 (0.038)
(3) pHEMA mixed with lysozyme before film formation	0.47 (0.076)	0.39 (0.89)	0.19 (0.068)

reduction in the constituents (0.06% pHEMA) could not account for the near tenfold decrease in $C_6H_9O_2^+$ ion intensity from Figs. 2(a)–2(c). Quantitative comparisons between samples are therefore not readily possible.

An enriched lysozyme surface layer is observed when lysozyme is combined as a component of the dip coating solution signifying that lysozyme diffuses toward the hydrogel–water interface, presumably as a result of leaching into the water.

In the pure pHEMA sample, complementary pHEMA depletion and water enrichment is indicated by the characteristic ion trends with etch time [Fig. 2(a)]. Such complementary and self-consistent trends are not seen in from the samples with lysozyme [Figs. 2(b) and 2(c)], suggesting that this incorporation induces “surface transience” supporting the view of a significant role for the matrix effect related to lysozyme presence. Recent studies using ToF-SIMS depth profiling illustrate the complexity of this effect when depth profiling organic materials.³⁰

V. CONCLUSION

A sample preparation procedure using argon gas in an “air gun” style to remove the surface frost accumulated on the cooled sample surface on exposure to ambient atmosphere yielded interesting results. It has been demonstrated that poly(2-hydroxyethyl methacrylate) hydrogels incorporating lysozyme can be successfully chemically characterized as frozen hydrated films by utilizing 10 keV Ar_{1500}^+ cluster sputtering by ToF-SIMS. The methodology allows full through-thickness chemical characterization of the pHEMA hydrogel by ToF-SIMS using the $C_4H_6N_2^+$ secondary ion to track lysozyme distribution through the hydrogels.

Lysozyme was found to be sufficiently mobile within the hydrated hydrogel film to diffuse into the films from solution. When lysozyme is combined with pHEMA into the dip coating solution, it diffuses out of the hydrogel resulting in an increased loading at the surface.

This method of 3D chemical characterization of frozen hydrated samples is of particular importance as it provides a way of effective full through-thickness chemical characterization of hydrogels, which is of importance to the biomedical research field.

ACKNOWLEDGMENTS

This work was supported by the EPSRC CTD in Regenerative Medicine. The authors thank Taranjit Singh

and Sebastiaan Van Nuffel for consultation on film preparation and ToF-SIMS, as well as Sunil Rajput for editing of the preparatory methodology. Finally, they thank Nottingham University and The Laboratory of Biophysics and Surface analysis for their support.

- ¹M. P. Lutolf, *Nat. Mater.* **8**, 451 (2009).
- ²S. C. Lee, I. K. Kwon, and K. Park, *Adv. Drug Delivery Rev.* **65**, 17 (2013).
- ³S. Kobel and M. P. Lutolf, *Curr. Opin. Biotechnol.* **22**, 690 (2011).
- ⁴Y. Mei, *Nat. Mater.* **9**, 768 (2010).
- ⁵M. P. Lutolf, P. M. Gilbert, and H. M. Blau, *Nature* **462**, 433 (2009).
- ⁶S. Khetan, M. Guvendiren, W. R. Legant, D. M. Cohen, C. S. Chen, and J. A. Burdick, *Nat. Mater.* **12**, 458 (2013).
- ⁷J. Zhu, *Biomaterials* **31**, 4639 (2010).
- ⁸D. S. W. Benoit, M. P. Schwartz, A. R. Durney, and K. S. Anseth, *Nat. Mater.* **7**, 816 (2008).
- ⁹W. L. Murphy, T. C. Mcdevitt, and A. J. Engler, *Nat. Mater.* **13**, 547 (2014).
- ¹⁰M. J. Dalby, N. Gadegaard, and R. O. C. Oreffo, *Nat. Mater.* **13**, 558 (2014).
- ¹¹A. D. Celiz, *Nat. Mater.* **13**, 570 (2014).
- ¹²C. A. DeForest and K. S. Anseth, *Annu. Rev. Chem. Biomol. Eng.* **3**, 421 (2012).
- ¹³B. N. Brown, C. A. Barnes, R. T. Kasick, R. Michel, T. W. Gilbert, D. Beer-Stolz, D. G. Castner, B. D. Ratner, and S. F. Badylak, *Biomaterials* **31**, 428 (2010).
- ¹⁴A. Ranga, S. Gobaa, Y. Okawa, K. Mosiewicz, A. Negro, and M. P. Lutolf, *Nat. Commun.* **5**, 1 (2014).
- ¹⁵N. Winograd, *Surf. Interface Anal.* **45**, 3 (2013).
- ¹⁶D. Rading, S. Ray, L. Yang, and A. G. Shard, *Phys. Chem.* **114**, 769 (2010).
- ¹⁷A. G. Shard *et al.*, *Anal. Chem.* **84**, 7865 (2012).
- ¹⁸J. Bailey, R. Havelund, A. G. Shard, I. S. Gilmore, M. R. Alexander, J. S. Sharp, and D. J. Scurr, *ACS Appl. Mater. Interfaces* **7**, 2654 (2015).
- ¹⁹M. A. Robinson and D. G. Castner, *Biointerphases* **8**, 15 (2013).
- ²⁰A. Piwowar, J. Fletcher, N. Lockyer, and J. Vickerman, *Surf. Interface Anal.* **43**, 207 (2011).
- ²¹M. Dickinson, P. J. Heard, J. H. A. Barker, A. C. Lewis, D. Mallard, and G. C. Allen, *Appl. Surf. Sci.* **252**, 6793 (2006).
- ²²T. Masumi, Y. Matsushita, D. Aoki, R. Takama, K. Saito, K. Kuroda, and K. Fukushima, *Appl. Surf. Sci.* **289**, 155 (2014).
- ²³K. Kuroda, T. Fujiwara, T. Imai, R. Takama, K. Saito, Y. Matsushita, and K. Fukushima, *Surf. Interface Anal.* **45**, 215 (2013).
- ²⁴A. P. Nair, B. J. Tyler, and R. E. Peterson, *Appl. Surf. Sci.* **231–232**, 538 (2004).
- ²⁵A. M. Piwowar, J. S. Fletcher, J. Kordys, N. P. Lockyer, N. Winograd, and J. C. Vickerman, *Anal. Chem.* **82**, 8291 (2010).
- ²⁶J. Lhoest, M. S. Wagner, C. D. Tidwell, and D. G. Castner, *Biomed. Mater.* **57**, 432 (2001).
- ²⁷C. Bradish, C. Brain, J. Messrs, and E. Hall, *Nature* **159**, 28 (1947).
- ²⁸M. Gemmei-Ide and H. Kitano, *J. Phys. Chem. B* **112**, 13499 (2008).
- ²⁹K. Lewis and B. Ratner, *J. Colloid Interface Sci.* **159**, 77 (1993).
- ³⁰A. G. Shard, S. J. Spencer, S. A. Smith, R. Havelund, and I. S. Gilmore, *Int. J. Mass Spectrom.* **377**, 599 (2015).