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A new particle mounting method for surface analysis

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The chemical analysis of microparticles is challenging due to the need to mount the particles on a substrate for analysis; double-sided adhesive tape is often used (sometimes conductive), however that is usually coated with poly (dimethyl siloxane) (PDMS) that is often used as a release agent. PDMS is a common surface contamination that can mask surface chemistries and hinder material performance where it is dependent on this contaminated interface. It is known that PDMS contains a very mobile oligomeric fraction that readily diffuses across surfaces resulting in the contamination of mounted particulate samples before and during surface chemistry analysis. This makes it impossible to determine whether the PDMS has arisen from the analysis procedure or from the sample itself. A new sample preparation method is proposed where polymer microparticles are mounted on a poly (hydroxyethyl methacrylate) (pHEMA) polymer solution, which we compare with particles that have been mounted on adhesive discs using time-of-flight secondary ion mass spectrometry (ToF-SIMS) and 3D OrbiSIMS analysis. Particles mounted on the pHEMA substrate results in a reduction of PDMS signal by 99.8% compared with microparticles mounted on adhesive discs. This illustrates how a simple, quick and inexpensive polymer solution can be used to adhere particles for analysis by ToF-SIMS, or other surface chemical analysis techniques such as X-ray photoelectron spectroscopy (XPS), without introduction of large amounts of silicone contaminant.

KEYWORDS
microparticle analysis, PDMS-free, sample preparation, ToF-SIMS

1 | INTRODUCTION

Cell-instructive polymers have been shown to modulate a range of surface/cell interactions including attachment, proliferation and differentiation in planar but also particle formats.[1–6] These rely on the surface chemistry of materials influencing biological function, through interactions with the polymers and the complex bio-interface formed by molecules adsorbed from the cell culture media or biological milieu in vivo. Consequently, producing and verifying defined biomaterial surface chemistries are of importance in design and production of medical devices.[7,8] Contamination on the surface of biomaterials can alter performance,[9] and detailed analysis of the appearance of poly (dimethyl siloxane) (PDMS) at surfaces has been made with techniques such as X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS).[10,11] Due to the mobility of PDMS oligomers, surface coatings have been shown to become coated by silicone molecules over time.[12–14] Specifically, the physicochemical properties of PDMS have been shown to strongly affect cell attachment.[15,16] The hydrophobicity of PDMS has been suggested to promote the non-specific

adsorption of biomolecules that also may affect cellular behaviour.[16,17]

Surface chemistry plays a critical role dictating the cell response to surfaces, and polymer particles have recently been shown to be able to control complex biological function by using bespoke surfactants.[4] As a practical response to the need to mount particulate samples for analysis, a common method employs double-sided adhesive tapes such as carbon adhesive discs and double-sided adhesive tape that utilise release layers of PDMS, which can then migrate onto the mounted particles.[18] This then makes it impossible to determine whether PDMS arises from the sample manufacture or from the mounting discs. Post-treatment of samples with solvents or techniques such as ultraviolet-ozone treatment have been used previously to remove PDMS,[14] but they are unsuitable as they could also affect the surface chemistry of samples; therefore, a different approach is required. Dry-adhesive solutions have been developed such as the Gecko tape that uses carbon nanotubes to promote adhesion without needing an adhesive layer.[11,19] Although effective, this solution is costly and less convenient compared with a readily available homemade substrate. Alternative solutions including pressing powders into surfaces such as indium and also sputtering processes from the analytical equipment have also been used.[20] To avoid the need for mechanical force to embed particles onto a surface or using a sputter cleaning process in which the native surface of the samples could be significantly changed, here we explored a sample preparation method that used 4% w/v poly (hydroxyethyl methacrylate) (pHEMA) in a 95:5 v/v ethanol : water solution applied to a glass cover slip as a substitute for an adhesive disc. Sprinkling the particles on top of this as it is drying allows them to be held onto the surface. pHEMA was chosen as the substrate due to a relatively high solubility in ethanol compared with many polymers. Ethanol was chosen as the solvent as it is a highly volatile solvent and the polymer particles used in this report are insoluble in ethanol, ensuring that the mounting method would not disrupt or damage the particles prior to analysis. By using a simple polymer solution, this also reduces the number of possible contaminations that could be introduced to the sample surface. However, as this approach is using the deposition of particles into pHEMA solution, it is intended for particle material surfaces that are chemically stable in liquids. This approach allowed for the analysis of particles under development to control biological function including reducing bacterial biofilm formation and promoting wound-healing behaviour by stimulating immune cells.

2 | MATERIALS AND METHODS

2.1 | Particle preparation

The polymer microparticles used in this study were prepared using droplet microfluidics with a photocured 1,6-hexanediol diacrylate (Sigma Aldrich) core with a hydroxy-3-phenoxypropyl acrylate-co-poly (ethylene glycol) methyl ether methacrylate (HPhOPA-co-mPEGMA) polymer surfactant decorating the surface.[4] Particles were then placed onto either a double-sided electrically conductive carbon-based adhesive disc (Agar Scientific Ltd) or a pHEMA solution to adhere particles onto a substrate that could be analysed.

2.2 | Sample preparation on pHEMA

Support substrates were prepared by dip-coating glass coverslips in a 4% w/v pHEMA (Sigma Aldrich) in 95:5 ethanol (Fisher Scientific) and deionised (DI) water solution. Manual dip-coating was used as it allowed a uniform layer of pHEMA to be placed onto the glass coverslip substrate. After dip-coating, coverslips were left for 2 min to allow partial evaporation of ethanol from the solution, at which point the particle powder was dropped onto the surface of the coated coverslip from a spatula. This allowed particles to adhere to the viscous liquid surface, removing the need to apply additional pressure after depositing particles on the substrate surface, as commonly utilised with adhesive tapes. The coverslips were then left at ambient conditions for 12 h to allow the residual ethanol and water to evaporate securing the particles in the pHEMA support. Shortly before insertion into the vacuum chamber for analysis by ToF-SIMS, any loose particles were removed with a jet of compressed nitrogen. This is a critical step to ensure no loose particles are removed by the vacuum pumps during evacuation of the spectrometer entry chamber, or by the voltage applied to the sample which would cause charged particles to be extracted from the sample and accumulate in the analyser extractor cone or the analyser/detector. This step is also employed when using adhesive discs. The overall sample preparation process is outlined schematically in Figure 1.

2.3 | Time-of-flight secondary ion mass spectrometry

A ToF-SIMS IV (IONTOF GmbH) instrument using a 25-keV Bi_3^+ primary ion source was used for the bulk of the analysis. Bi_3^+ primary ions were used with a target current of ~0.3 pA. Analysis for positive and negative spectra was acquired over a 500 × 500 μm scan area. Other analyses parameters were a cycle time of 100 μs, one shot/frame/pixel, one frame/patch and 20 scans per analysis. As the samples were of a non-conductive nature, charge compensation in the form of a low-energy (20 eV) electron flood gun was applied. Images and spectra were acquired using SurfaceLab 6 software and analysed using SurfaceLab 7.1 software. Internal mass calibration was performed using CH_3^+, $C_2H_3^+$, $C_4H_5^+$ and $C_5H_9^+$. Images were acquired and analysed using SurfaceLab 7.1 software.

2.4 | 3D OrbiSIMS images

The IONTOF Hybrid SIMS instrument was used to acquire ToF-SIMS images in the delayed extraction mode. A 30-keV pulsed Bi_3^+ ion beam was used as the analysis beam with an analysis area of

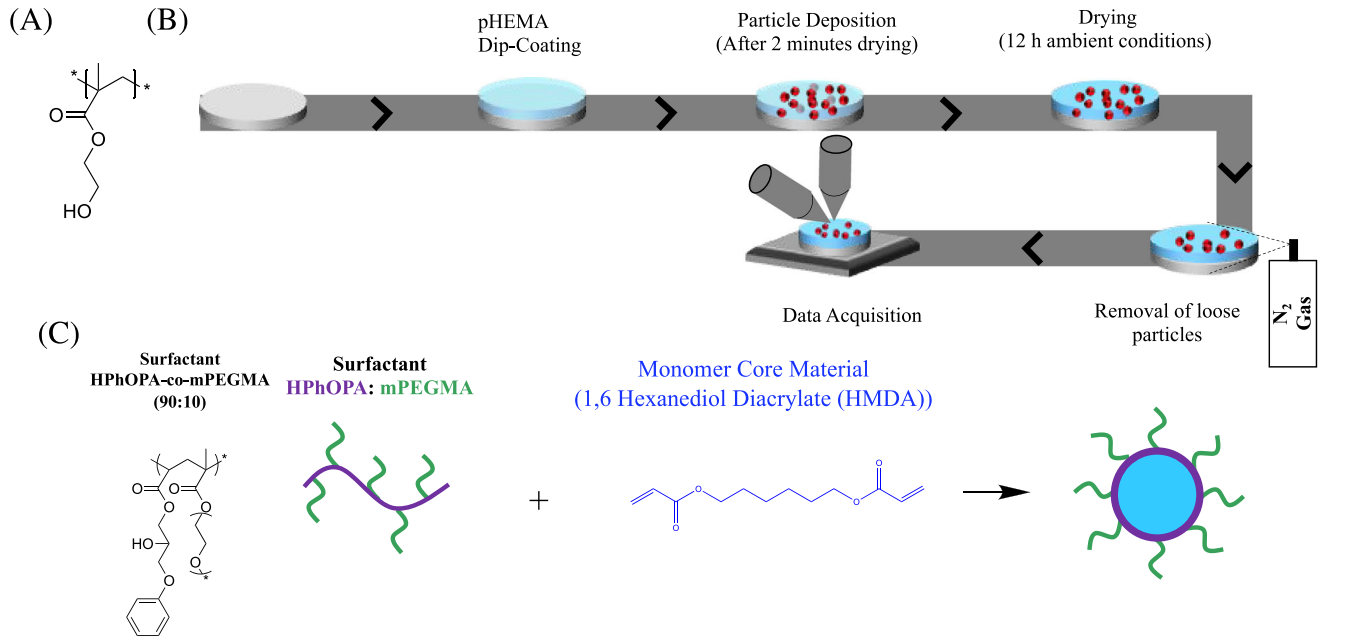

FIGURE 1 Schematic of process. (A) Chemical structure of poly (hydroxyethyl methacrylate) (pHEMA) that is turned into a solution by solubilising 4% (w/v) pHEMA in 95:5% (v/v) ethanol : water. (B) Microparticle sample preparation method demonstrating a glass coverslip being coated in a pHEMA solution prior to depositing particles on the surface. The coverslips are then left for 12 h in ambient conditions for ethanol to evaporate from the sample surface. Loose particles are then removed using nitrogen gas. Samples are then placed into a vacuum chamber for time-of-flight secondary ion mass spectrometry (ToF-SIMS) analysis. (C) Production method of polymer microparticles with 1,6-hexanediol diacrylate cores and a polymer surfactant surface: HPhOPA-co-mPEGMA[4]

300 × 300 μm. Other analyses parameters were a cycle time of 150 μs, an analyser extraction delay of 0.085 μs, one shot/frame/pixel, one frame/patch and 20 scans per analysis. Charge compensation was done with a low-energy electron flood gun (20 eV). Data were acquired in positive polarity. Internal mass calibration was performed using $C_2H_3^+$, $C_3H_5^+$, $C_4H_7^+$ and $C_7H_7^+$. Images were acquired and analysed using SurfaceLab 7.1 software.

2.5 | Profiles mode

Calibration of the Orbitrap analyser was performed on a silver plate, following the method described by Passarelli et al.[21] The Bi_3^+ liquid metal ion gun with a beam of 400 μm and 20% long pulses were employed for calibration together with the ThermoFisher Tune software.

For the acquisition of 3D OrbiSIMS profiles, a 20-keV Ar_{3000}^+ analysis beam of 20-μm diameter was used as primary ion beam. Duty cycle was set to 4.4%, and GCIB current was ~300 pA. The Q Exactive data acquisition was run on the area of 300 × 300 μm using random raster mode with crater size 381.9 × 381.9 μm. The cycle time was set to 200 μs. Optimal target potential varied for different samples, oscillating at approximately −195 V. Argon gas flooding was in operation to aid charge compensation, and pressure in the main chamber was maintained at 9.0×10^{-7} bar. Depth profiles were collected in positive and negative polarity, with a mass range of 50–750 *m/z*. The injection time was set to 500 ms. Mass-resolving power was set to 240,000 at 200 *m/z*. Two hundred scans were conducted. 3D OrbiSIMS data were acquired and analysed using SurfaceLab 7.1 software.

3 | RESULTS AND DISCUSSION

To compare the use of the pHEMA support substrate to adhesive discs, they were first analysed using ToF-SIMS to identify any evidence of PDMS without the addition of particles. The secondary ion peaks $SiC_3H_9^+$ (*m/z* 73.0435), $Si_2C_5H_{15}O^+$ (*m/z* 147.0701), $Si_3C_5H_{15}O_3^+$ (*m/z* 207.0218), $Si_3C_7H_{21}O_2^+$ (*m/z* 221.0992) and $Si_4C_7H_{21}O_4^+$ (*m/z* 281.0369) originate from PDMS.[10,22] The assignment and mass stated of peaks are as determined by SurfaceLab software and relate directly to the identified peaks in the spectra. Other high intensity peaks were also identified, and peaks were chemically assigned with a deviation of less than 100 ppm (Figure 2).

The characteristic PDMS peaks were identified within the adhesive disc substrate as expected. This confirmed the adhesive discs as a source of PDMS contmation. A small amount of PDMS was also found on the dip-coated pHEMA substrate. In order to confirm unique ions associated to the pHEMA substrate, pHEMA was compared with a 2D film produced from the polymer surfactant poly(HPhOPA-co-mPEGMA) used to manufacture the particles. The ions $C_4H_5O^+$ and $C_8H_9O_3^+$ were shown to be unique to pHEMA

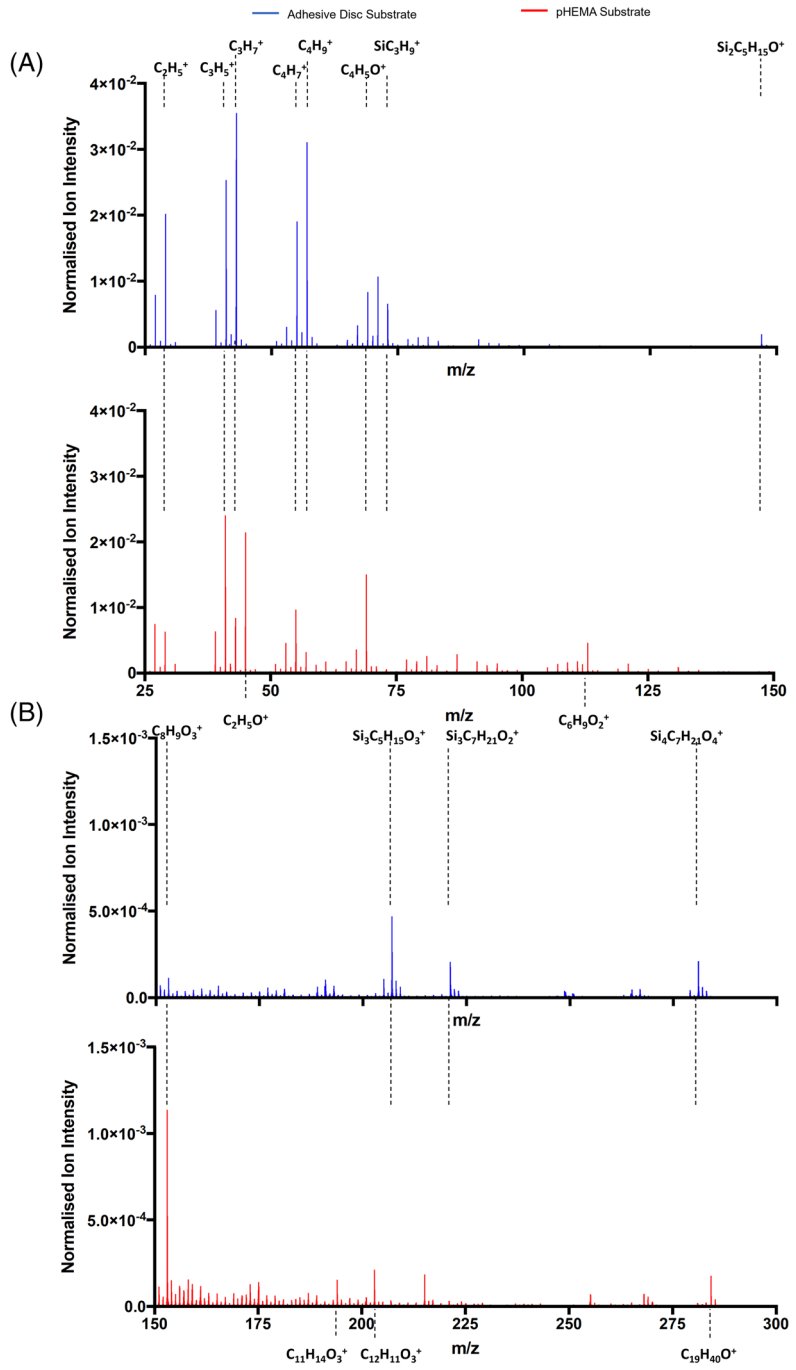

FIGURE 2 Positive time-of-flight secondary ion mass spectrometry (ToF-SIMS) spectra comparing two substrates: adhesive disc and pHEMA. Adhesive disc sample traces are in blue, and the pHEMA substrate spectra trace in red. (A) Positive spectra in the *m/z* range of 25–150 and (B) positive spectra in the *m/z* range of 150–300. High intensity and peaks of interest have been labelled. (C) Table showing identified key ions and associated deviation, where the deviation is the value in ppm, which the assigned mass is from the accurate theoretical mass

(C)

Mass (m/z)	Assignment	pHEMA Deviation (ppm)	Adhesive Tape Deviation (ppm)	Description
45.0343	$C_2H_5O^+$	17.3	7.6	pHEMA unique peak
69.0338	$C_4H_5O^+$	4.4	9.8	Common high intensity peak
73.0435	$SiC_3H_9^+$	-44.7	-21.1	PDMS contamination peak
113.0618	$C_6H_9O_2^+$	18.7	14.6	pHEMA unique peak
147.0701	$Si_2C_5H_{15}O^+$	30.5	17.0	PDMS contamination peak
153.0664	$C_8H_9O_3^+$	77.3	47.6	pHEMA unique peak
194.1000	$C_{11}H_{14}O_3^+$	32.1	-2	pHEMA unique peak
203.0845	$C_{12}H_{11}O_3^+$	70.2	70.9	pHEMA unique peak
207.0218	$Si_3C_5H_{15}O_3^+$	-50.9	-47.3	PDMS contamination peak
215.1854	$C_{16}H_{23}^+$	27.8	13.4	pHEMA unique peak
221.0992	$Si_3C_7H_{21}O_2^+$	67.2	99.6	PDMS contamination peak
281.0369	$Si_4C_5H_{15}O_4^+$	-50.7	-44.9	PDMS contamination peak
284.3189	$C_{19}H_{40}O^+$	40.6	8.3	pHEMA unique peak

when compared with the 2D polymer film of the functional surface (Figure S1). These ions were also used to observe pHEMA coverage of particles and showed to have a 95% reduction in pHEMA signal, suggesting minimal coverage of pHEMA (Figure S2). Figure 2 also shows that traces of PDMS were seen in the pHEMA substrate, which was not ideal, but is an order of magnitude less than found on the adhesive discs. To determine the source of the PDMS, pHEMA powder was isolated using cyanoacrylate adhesive and analysed on aluminium foil, which showed an increase in PDMS signal compared with the adhesive (Figure S3). This was also observed when the pHEMA solution was coated on aluminium foil compared with uncoated foil (Figures S4–S5).

ToF-SIMS has a relatively poor resolution, and a single ToF-SIMS peak could contain multiple assignments. One such fragment, $C_3H_5O_2^+$ (73.0287 u), which is a unique ion for pHEMA appears close to the $SiC_3H_9^+$ (73.0435 u) PDMS peak. Additionally, due to the shape of microparticles, ToF-SIMS peaks can be further broadened by their different flight distances, which caused a significant overlap between the $C_3H_5O_2^+$ (73.0287 u), and $SiC_3H_9^+$ (73.0435 u), peak (Figure S6). This effect can be partially solved by using region of interest (ROIs) analysis to mitigate the topography by selecting areas from similar heights. However, an approach that has an improved peak resolution would be advantageous. To achieve this, the 3D OrbiSIMS instrument was used.[21] To observe if any PDMS oligomers had

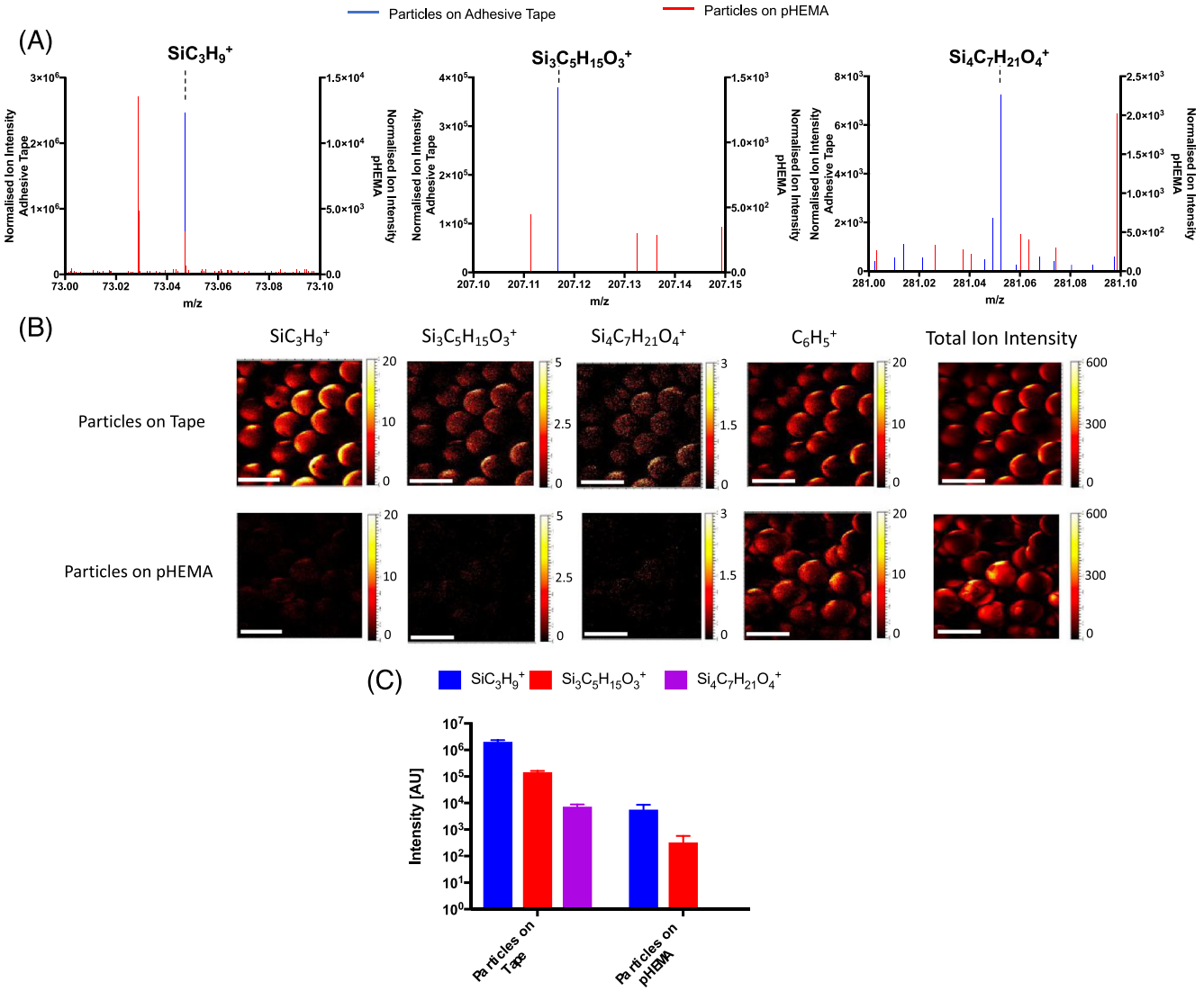

FIGURE 3 3D OrbiSIMS analysis of polymer microparticles mounted on adhesive discs and pHEMA. Characteristic PDMS peaks $SiC_3H_9^+$, $Si_3C_5H_{15}O_3^+$ and $Si_4C_7H_{21}O_4^+$ were identified on both mounting methods and used to observe the effect of substrate on particle sample contamination. (A) Individual spectra of characteristic PDMS peaks obtained using OrbiTrap™ analyser on 3D OrbiSIMS to obtain increased peak resolution. (B) Chemical images were obtained with ToF-SIMS modality and used to show the distribution of PDMS ions on the surface of particles. $C_6H_5^+$ (pHPhOPA-co-mPEGMA) was used to identify the chemistry on the surface of the particles. Scale bars are 100 μm. (C) Quantification of data for $SiC_3H_9^+$, $Si_3C_5H_{15}O_3^+$ and $Si_4C_7H_{21}O_4^+$ ($N = 3$). PDMS, poly (dimethyl siloxane); pHEMA, poly (hydroxyethyl methacrylate)

transferred to particles under analysis, particles were mounted on both the adhesive discs and on pHEMA as illustrated in Figure 1. The samples were then analysed using the 3D OrbiSIMS to observe the difference in PDMS inclusion on the surface. Characteristic ions for PDMS [$SiC_3H_9^+$(73.0471 u), $Si_3C_5H_{15}O_3^+$ (207.0322 u) and $Si_4C_7H_{21}O_4^+$(281.051 u)] were identified via spectra, secondary ion images and secondary ion intensities as shown in Figure 3. The silicone was observed uniformly on the adhesive disc mounted particles but at a far lower level and unevenly on the pHEMA mounted particles. Total ion secondary ion images as well as a unique chemical identifier for the polymer surface [$C_6H_5^+$ (77.0356 u)], are also included. Full spectra comparison between *m/z* 50–300 can be seen in Figure S7.

By mounting particles in pHEMA, a large reduction in the amount of PDMS was observed on the particle surfaces, the underlying pHEMA minimally contributed to the spectrum, with an average PDMS peak reduction of 99.8 ± 0.1% over the total imaged area compared with mounting on an adhesive disc alternative. Comparing this to the substrates without particles which exhibited a differential in PDMS signal intensity of 27%, this suggests that the low levels of silicone on pHEMA do not diffuse on the surface uniformly (Figure S8). The analysis has shown that there are only trace amounts of PDMS visible at the surface when using pHEMA as a substrate, demonstrating no dominating surface contamination on these particles that could affect subsequent biological experiments. As PDMS oligomers are known to have a high mobility, this would suggest that samples on the surfaces would become contaminated over time.[14] Further analysis showed successful identification of particle surface ($C_6H_5^+$) for both analysis methods (Figure S9). Therefore, by changing the substrate from the adhesive discs to pHEMA has not affected the analysis method's ability to determine the surface chemistry of the polymer microspheres. This sample preparation step has introduced an additional quality control check in the manufacturing process of polymer microparticles, which has increased the accuracy of subsequent biological assays by ensuring a high quality of material is produced for assessment.

4 | CONCLUSION

In conclusion, a sample preparation method has been developed for mounting polymer microparticles for surface analysis that could be applied to other particulate samples. To demonstrate this method, particles with a functional surface were analysed on both a pHEMA substrate and an adhesive disc substrate. The pHEMA sample preparation method was shown to prevent the inclusion of PDMS on the surface of particles by 99.8 ± 0.1% compared with adhesive discs. By using this method, it is possible to identify that no PDMS is introduced in the production process of polymer microparticles, which was previously not possible due to PDMS contamination commonly found on adhesive discs used for mounting samples for analysis. This approach could enable other surface chemistry techniques to mount samples for analysis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All relevant data are available from the University of Nottingham's Research Data Management Repository.

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