Solid-material-based coupling efficiency analyzed with time-of-flight secondary ion mass spectrometry

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The coupling behavior of a microparticle embedded amino acid active-ester into a Poly(ethylene glycol)methacrylate-film, synthesized onto a silicon wafer by a grafting from approach, is characterized using dynamic time-of-flight secondary ion mass spectrometry (ToF-SIMS) to analyze the 3d distribution of the amino acids in the polymer film. Besides standard solid phase peptide synthesis, employing solubilized amino acids in a solvent, we used solid polymer microparticles, incorporating the amino acids. These microparticles were especially designed for a new technique to produce high-density combinatorial peptide microarrays: upon heating, the particles become viscous, which releases the embedded amino acids to diffuse and couple to the surface. In the scope of the development of this new particle-based application, ToF-SIMS is used to analyze a complex chemically modified polymer surface layer. Due to depth profile measurements, it is possible to investigate the particle-based coupling reaction not only on the surface, but also into the depth of the PEGMA film.

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1. Introduction

In 1992 Ronald Frank developed the SPOT-Technique, an automated approach for a full combinatorial peptide synthesis in the array format on a membrane support [1,2]. This procedure is based on the concept of the solid-phase peptide synthesis (SPPS), which was invented by Bruce Merrifield in 1963 [3]. Today, the SPOT-Technique (see Fig. 1a) or variants thereof are commercially applied by companies like e.g. INTAVIS Bioanalytical Instruments AG [4], JPT Peptide Technologies GmbH [5], and others [6–8]. The combinatorial diversity arising from the 20 different amino acids is the major challenge for in situ peptide array synthesis. Meanwhile several research groups have investigated and reported novel strategies for the production of high-density peptide arrays [9]. Lithographic approaches, combining photomasks with photo-labile protecting groups [10,11] or more advanced technologies using digitally controlled patterned illumination to generate acids for deprotection by photolytic reactions [12] represent applied synthesis pathways. In 2007 and 2008, two straightforward xerographic methods to produce high-density combinatorial peptide microarrays were presented [13,14]. These techniques use polymer–based, amino acid loaded microparticles, which are addressed to a functionalized, Poly(ethylene glycol) methacrylate-(PEGMA) coated support [15,16] by electromagnetic fields (see Fig. 1b). The microparticles' hull consists of a commercially available polymer that was developed for toner particles in xerographic applications [14]. Microparticles are produced by dissolving one type of amino acid derivative and the polymer matrix material in a solvent, which is then evaporated in a spray dryer. The resulting polymer microparticles embed the amino acid derivatives. This material forms a brittle solid at room temperature but turns viscous at elevated temperatures. The viscous polymer efficiently confines the material to very small reaction sites, but at the same time frees the embedded amino acid derivatives to diffuse and couple to the surface (see Fig. 1c). Custom-made xerography toner-batches, one for each of the 20 amino acids, were developed and characterized to produce peptide microarrays. Towards the
Fig. 1. Combinatorial synthesis of peptides. Amino acids are deposited on a surface with (a) the SPOT technology or (b) the particle-based technology. Activated amino acids in a solvent directly couple to the surface upon deposition, whereas solid microparticles have to be heated, to induce the coupling reaction. (c) The coupling reaction is performed in four steps: (1) deposition and (2) coupling of amino acids to amino groups, (3) blocking of unreacted amino groups and, (4) removing the amino-terminal protecting groups.

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development of very high-density peptide arrays, Maerke et al. successfully advanced this technology by combining the xerographic particle approach with advanced laser processing. This method, called Combinatorial Laser Fusing, allows for the synthesis of high-density peptide microarrays with a spot size of 10 µm [17,18].

Despite modern surface-sensitive spectroscopic techniques like IR- or XP-spectroscopy it is still challenging to analyze chemically modified thin films like the polypyrrole coated silicon supports presented herein. We use ToF-SIMS as the method of choice to characterize the composition and the lateral distribution of characteristic moieties of our surfaces and thin films [19,20]. Today, the application range of ToF-SIMS includes engineering applications as well as the analysis of biological and organic samples [19,21–23]. While surface mapping of organic chemistries is usually readily achieved, molecular depth profiling and 3D imaging developed later [24] and gained strong attraction not before the development of polyatomic sputter ions like C60 and, more recently, Ar clusters, but still remain significant analytical challenge [25]. Table 1 lists the most important aspects of 3D imaging of organic layers based on dual beam depth profiling in SIMS. Apart from the applied technologies alternative approaches are given. It should be noted, that recently data of the detailed “VAMAS” multi-laboratory study were published: Layered samples of Irganox 1010 and either Irganox 1098 or Fmoc-parafluoro-1-phenylalanine were used and analysis was performed using Ar cluster ion sputtering and either X-ray photoelectron spectroscopy (XPS) or ToF-SIMS [26].

2. Materials and methods

2.1. Materials

The chemicals and solvents used for the experiments were purchased from the following suppliers: 2,2'-Bipyridine (Bipy) (>99%), molar sieves (3 Å, 4 Å) from Alfa Aesar (Karlsruhe/Germany); Fmoc-β-alanine-(OH) from Iris Biotech (Marktredwitz/Germany); Chloroform (pro analysis, p.a.), Dichloromethane (DCM) (p.a.), N,N-Diisopropylethylamine (for synthesis), N,N-Dimethylformamide (DMF) (p.a.), Hydrogen peroxide (H2O2) (30%) (for synthesis), Sulfuric acid (H2SO4) (for synthesis), Isopropyl alcohol (PrOH) (p.a.), Potassium hydroxide (KOH) (p.a.), Methanol (p.a.), Piperidin (for synthesis) from Merck (Darmstadt/Germany); (3-Amino)propyltriethoxysilane (APTES) (>99%), Copper(I) bromide (Cu(I)Br) (>98%), N,N'-Diisopropylcarbodiimide (>99%), Fmoc-Cys(Tri)-OPfp (>98%), 1-Methylimidazole (NMI) (>99%), α-Bromoisobutryl-bromide (98%), Ethanol (>99.5%), Acetic anhydride (>98%) from Sigma Aldrich Chemie GmbH (Steinheim/Germany); S-LEC-P LT 7552 from Sekisui Chemical CO, LTD. (Osaka/Japan); graphite nanoparticles (99.9%, <30 nm, Tm = 3652 °C) from MIT Corp. (Richmond, CA/USA).

2.2. Microparticle production

8.8 g of the polymer matrix material S-LEC-P LT 75 52 (Sekisui/Japan) and 0.2 g graphite nanoparticles were dissolved/suspended in 100 ml dichloromethane (p.a.) by vigorous stirring. After the polymer was dissolved and the nanoparticles were homogeneously dispersed, 1.0 g of Fmoc-Cys(Tri)-OPfp was added and dissolved. The solution/dispersion was introduced into a B-290 spray-dryer (in combination with the B-295 Inert-Loop, which allows spray drying of organic solvents) over a silicon tube. The spray-dryer and the Inert-Loop were purchased from Büchi Labortechnik GmbH (Esen/Germany). After the spray drying process was completed, the resulting particles were taken from the collecting vessel.

2.3. Surfaces and solid supports

Silicon wafers served as solid supports. After 30 min of cleaning with 30% (v/v) hydrogen peroxide (30%) in concentrated sulfuric acid in a petri dish under constant shaking, the cleaning solution was carefully decanted and the surface was rinsed with Millipore water and ethanol (p.a.) and dried in an airstream.

Immediately after drying, the silicon wafer was covered with 10 ml of a solution of 290 µl (3-Amino) propyltriethoxysilane (APTES) and 240 µl Millipore water in ethanol (p.a.) and incubated for 16 h inside a desiccator under argon-atmosphere. The decanting of the APTES-solution has to be carried out under constant, thoroughly rinsing with ethanol (p.a.). A curing of the surface while rinsing, might lead to crystallization of non-reacted APTES traces that could damage the APTES-layer on the surface. Afterwards, the wafer was washed with ethanol (p.a.)(3 × 3 min) on a shaker, dried in an airstream and tempered in an oven at 120 °C for 45 min to complete the condensation reaction.

A 0.6 mM N,N-Diisopropylethylamine (DPEA) solution in dry dichloromethane (DCM) (stored above 4 Å molar sieve) was provided in a Schlenk-flask under argon atmosphere and cooled down to 0 °C. Under vigorous stirring, a 0.2 mM α-Bromo isobutryl bromide solution in dry DCM (stored above 4 Å molar sieve) was added via a dropping funnel under argon flow. The APTES-functionalized wafer was covered by the reaction mixture, placed inside a petri dish in the desiccator. After 16 h of incubation at room temperature under argon, the reaction solution was decanted and the surface was washed on a shaker with dry DCM (2 × 3 min) and methanol (p.a.) (2 × 2 min) and dried in an airstream.

All following steps were performed under argon flow. A solution of 10 ml PEGMA, 10 ml Millipore water and 10 ml methanol (p.a.) was set up inside a Schlenk-flask, which was evacuated three times and put under argon atmosphere before. 280 mg of 2,2'-Bipyridine (Bipy) were added to the solution and dissolved under stirring. After the Bipy was dissolved completely, 128 mg Cu(I)Br were added and the reaction mixture was stirred for another 5 min. The functionalized surface was incubated in the PEGMA solution inside a petri dish in the desiccator for 16 h under argon atmosphere at room temperature. After the incubation was complete, the surface was rinsed with Millipore water and washed with Millipore water (3 × 5 min) and methanol (2 × 3 min) on a shaker and dried in the airstream. In the case of remaining residua from the polymerization reaction, the surface was cleaned in chloroform by sonication (<30 s).

2.4. Fmoc-β-alanine functionalization of the PEGMA film

1.2 equivalents of N,N'-Diisopropyl-carbodiimide (DIC) were added to a 0.2 M solution of Fmoc-β-alanine in dry DMF (stored above 3 Å molar sieve) inside a Schlenk-flask under argon with a syringe via a septum. After stirring for 5 min at room temperature, 2.0 equivalents of 1-Methylimidazole (NMI) were added and the reaction mixture was stirred for another minute. The PEGMA film on top of the silicon wafer was covered with the DMF solution inside a petri dish, placed in a desiccator under argon flow. The closed desiccator has now been evacuated and filled with argon three times. The incubation was performed overnight, while coupling to hydroxyl-groups. Because of the higher nucleophilicity, 2 h of incubation was sufficient for coupling to amino-functions. After the coupling reaction was completed, the solid support was washed with DMF (p.a.) (3 × 5 min) and methanol (2 × 3 min) on a shaker, dried in an airstream and stored at 4 °C under argon until further use.
2.5. Blocking

After each coupling reaction on the solid support, non-reacted functional groups were acetylated by covering the PEGMA film with a solution of 1 ml acetic anhydride, 2 ml DIPEA in 7 ml DMF (p.a.). After this, the solid support was washed with DMF (p.a.) (3 × 5 min) and methanol (2 × 3 min) on a shaker, dried in an airstream and stored at 4 °C under argon until further use.

2.6. Removal of the Fmoc-protecting group

The Fmoc-protecting group was removed by covering the PEGMA film on the silicon wafer with a 20% (v/v) piperidine solution in DMF at room temperature for 20 min. Reaction yields of the Fmoc-β-alanine coupling steps were controlled by photometric determination of the concentration of the piperidyl-dibenzofulvene adduct inside the deprotection solution. After deprotection, the solid support was washed with DMF (p.a.) (3 × 5 min) and methanol (2 × 3 min) on a shaker, dried in an airstream and stored at 4 °C under argon until further use.

2.7. Particle-based coupling reaction

The microparticles were deposited on the synthesis surface (6.25 mg/cm²) with a spatula. Subsequently, the surface was heated...
in a petri-dish in the oven for 90 min at 90 °C under argon. After the sample was cooled down to room temperature, non-reacted amino acid and other particle components remaining on the synthesis surface were quickly rinsed away with DCM (p.a.). Afterwards, the surface was washed with DCM (p.a.) (3 × 5 min) and methanol (p.a.) (2 × 3 min) in a petri-dish on a shaker and dried in an airstream.

### 2.8. Solution-based coupling reaction

The PEGMA film synthesized on top of a silicon wafer, functionalized with three β-alanine spacer-molecules was covered by a 0.2 M Fmoc-Cys(Trt)-OPfp solution in dry DMF (stored above 3 Å molar sieve) inside a petri-dish in a desiccator under argon flow. After 2 h of incubation at room temperature under argon, the PEGMA film was washed with DMF (p.a.) (3 × 5 min) and methanol.
instrument was able to scan a 500 × 500 µm² area with a lateral resolution of 4 µm. While measuring depth profiles, the surface was eroded by sputtering with 500 eV Cs⁺ or 20 keV C₆₀⁻ ions. The Cs gun was operated in interlaced mode, this means after each Bi pulse for mass spectrometry the Cs sputter beam was activated during each drift time of the secondary ions in the analyzer. The C₆₀ gun was operated in non-interlaced mode, this means several frames were taken with Bi primary ions for analysis followed by a 1.4 s C₆₀ erosion cycle and a 0.6 s pause for charge compensation. Surface charges were compensated by a 21 eV electron flood gun. C⁺, CH⁺, H₂⁺ and CH₃⁺ as well as C⁻, C₂⁻, C₃⁻ signals were used for calibration.

2.10. SEM images

The scanning electron microscope (SEM) images were obtained using a Supra 60 VP scanning electron microscope from Carl Zeiss GmbH (Jena/Germany). Prior to the SEM imaging the samples were coated with a 15 nm silver layer by a K575X Sputter Coater from Quorum Technologies Ltd (East Sussex/UK) using a 30 µm Ag-target, sputtering 3.5 min with 25 mA.

2.11. HPLC measurements

The comparative HPLC measurement was performed on an AKTA™ Purifier 10 from Amersham Pharmacia Biotech (Freiburg/Germany). The HPLC runs were carried out via a CC 125/3 Nukleosil 100-3 C18 HD column in combination with a CC 8/3 Nucleosil 100-3 C18 HD pre-column, both from Macherey–Nagel (Düren/Germany). A 0.05 M aqueous triethylammonium phosphate buffer (pH 2.25) served as Eluent A. Eluent B was a mixture of 10% (v/v) Eluent A in acetonitrile. The mobile phase was pumped with a flow rate of 0.5 ml/min and a gradient of 60–95% (v/v) referring to Eluent B in 30 min at 23 °C. The detection wavelength was 215 nm.

3. Results and discussion

For the experiments, a PEGMA film synthesized on top of a silicon wafer by surface-induced Atom Radical Transfer Polymerization (si-ATRP) served as solid support. To generate nucleophilic NH₂-groups inside the PEGMA film, the polymer was functionalized with three β-alanine spacer-molecules prior to the particle-based amino acid immobilization. To acquire a reliable ToF-SIMS depth profile, we introduced an amino acid derivative into the PEGMA film which yields strong and clearly assignable secondary ions, as outlined in Fig. 2.

The Fmoc-S-trityl-l-cysteine-pentafluorophenylester (Fmoc-Cys(Trt)-OPfp) was embedded into polymer microparticles by spray drying, using a B-290 Minispray-dryer in combination with...
an Inert-Loop B-295 (Büchi Labortechnik GmbH, Essen/Germany). Dichloromethane was used to dissolve and disperse the particle components homogeneously prior to the spray drying process. To determine the particle size distribution, a MASTERSIZER 2000 from Malvern Instruments (Worcestershire/UK) was used. A particle batch with a diameter size distribution showing the characteristic values δ(0.1) = 0.8 μm, δ(0.5) = 3.4 μm and δ(0.9) = 6.4 μm was produced by applying the spray drying parameters from Fig. 3. Differential scanning calorimetry measurements revealed that the microparticles undergo a glass transition at Tg = 65.6 °C, which is in accordance to the data published by Stadler et al. [114]. Comparative HPLC measurements validated the integrity of the Fmoc-Cys(Trt)-OPfp, embedded inside the microparticles (Fig. 4).

Equal amounts of particles were spread over comparably sized surfaces to produce samples for the ToF-SIMS measurements. The particle layers were heated at 90 °C under argon. Upon heating, the particle matrix material becomes viscous, converting into a gel-like state, and thus, mediates the coupling of the OPfp-activated cysteine derivative into the functionalized PEGMA film. After the particle-based coupling reaction, the solid support was washed thoroughly to remove non-reacted amino acid and remaining particle components from the polymer film. After drying the sample in an airstream, the ToF-SIMS measurements were performed.

First ToF-SIMS depth profiling results, obtained by using a 0.5 keV Cs+ ion beam to sputter the surface and 25 keV Bi+ ion pulses to analyze the secondary ions in interlaced mode, are shown in Fig. 5. The 79Br−-signal of the sample is shown in green. Br− can be found inside the functionalized polymer film on the silicon wafer, because the PEGMA film was synthesized by a grafting from Si-ATRP approach. Serving as a starting point for the ATRP reaction, α-Bromo-isobutylryl bromid was coupled to an (3-Aminopropyl) triethoxysilane (APTES) layer, immobilized on the silicon wafer surface. The 79Br− signal shows an accumulation of Br− in the upper region of the polymer film. A possible explanation for this concentration effect, considering the si-ATRP reaction mechanism, is the propagation of the PEGMA backbone which is associated with the bromide “growing out” of the polymer film [34–36].

Following a maximum, the 79Br− signal declines when reaching deeper regions of the polymer film. As soon as the sputter erosion removed the PEGMA film and reached the surface of the silicon wafer, the Si−-signal (Fig. 5, blue line) increases, while all other signals decline. With the side chain of the cysteine derivative, a sulfur heterocatom was introduced into the PEGMA film by particle-based immobilization. The development of the S−-signal (Fig. 5, black line) was monitored by the ToF-SIMS depth profile. Immediately before the Si−-signal gains intensity the S−-signal shows a local maximum. This observation can be referred to the forward implantation of individual sulfur atoms into deeper regions of the bulk of the PEGMA coating during Cs−-sputtering or changing matrix effects at the PEGMA/Si interface. The axis of abscessae represents a sputter ion fluence scale that cannot be directly converted into a depth scale. When sputtering two different material layers, as in our case (PEGMA film/silicon wafer), the linear correlation between sputter ion dose and depth of erosion is lost due to different sputter yields.

To reduce the forward implantation effects and the degradation of larger molecular structures ahead of the erosion front, a C60+ sputter beam was applied for molecular depth profiling. C60 is known to be less destructive during the sputter process [37–39]. The following investigation of a PEGMA film synthesized on a silicon wafer, functionalized with three β-alanine spacer-molecules, was performed before and after particle- and solution-based Fmoc-Cys(Trt)-OPfp immobilization. A blank PEGMA film with the spacer molecules served as reference. We compared the corresponding functionalized polymer film after coupling the Fmoc-Cys(Trt)-OPfp from solution with a similarly processed particle-based coupling sample. Solution-based immobilization was done by spreading a 0.2 M solution of Fmoc-Cys(Trt)-OPfp in N,N-Dimethylformamide (DMF) over the polymer film on the silicon wafer at room temperature under argon, while the particle-based coupling reaction was performed as described above. After carefully washing and drying the samples, ToF-SIMS measurements were performed. The depth profiles (Fig. 6) showed characteristic signals, clearly assignable to molecule-ions originating from the immobilized Fmoc-Cys(Trt).

Fig. 6 illustrates the CHS−-signal (m/z = 44.98) generated from the cysteine derivative’s side chain, the C6H5S+−-signal (m/z = 243.04) derived from the trityl side chain protecting group, and the C6H3S+−-signal (m/z = 165.07), attributed to the fragmentation of the N-terminal Fmoc-protecting group. For signals integrated over the whole depth of depth profile measurement, the highest intensities were always obtained from the sample prepared by solution-based Fmoc-Cys(Trt)-OPfp immobilization (Fig. 6, black lines).

Due to the nearly complete absence of other interfering signals (see green curves in Fig. 6), the trityl+−-signal appeared to be the best indicator for the characterization of the Fmoc-Cys(Trt)-OPfp immobilization inside the PEGMA film. Furthermore, the analysis of large (multi atomic) secondary ions eliminates forward implantation effects.

Fig. 7 shows the depth profile measurements on a functionalized PEGMA film after particle-based immobilization of Fmoc-Cys(Trt)-OPfp. This diagram shows the normalized intensities of the trityl+−-signal (Fig. 7, red data points), the CHS−- (Fig. 7, black data points) and the Si−-signal (Fig. 7, blue data points) plotted against the sputter ion dose density. As expected, the curve progression of the trityl+−-signal (Fig. 7, red data points) is in accordance with the CHS−-signal (Fig. 7, black data points) before reaching the surface of the silicon wafer. However, when the silicon wafer surface is reached – which is indicated by the gradually increasing Si−-signal (Fig. 7, blue data points) – the CHS−-signal unexpectedly seems to gain intensity. This behavior is caused by the mass overlap of the wafer specific SiOH− ion (m/z = 44.9802) and the CHS− side chain fragment (m/z = 44.9804). However, the very low Si− intensities found for low erosion depths indicate the absence of this cross talk within the polymer layer.

To assess the penetration-depth of Fmoc-Cys(Trt)-OPfp within the functionalized PEGMA film, trityl+−-signals of the two samples, coupled from solution vs. coupled from viscous particle matrix material, were compared (Fig. 8). Immobilization of the cysteine derivative to the PEGMA film from solution and particle matrix was performed as described above. Fig. 8 shows the determined ToF-SIMS depth profiles. To convert the sputter ion fluence into a depth scale, the depths of several sputter craters with known C60 ion fluences were measured by profilometry directly after the ToF-SIMS experiment [23].

While the trityl+−-signal of the solution-based immobilization (Fig. 8, black data points) is constant to a penetration-depth of 120 nm, the trityl+−-signal of the particle-based coupling reaction (Fig. 8, red data points) already starts to decline at roughly 60 nm. This depth does not correspond to the total thickness of the PEGMA layer (compare Si signal in Fig. 6). These results can be explained by the different properties of the used solvents: while PEGMA films show a good swelling behavior in DMF (at room temperature), the sterically demanding polymer chains of the viscous particle matrix material do not penetrate into deeper regions of the polymer film to mediate the coupling reaction (at 90 °C). Fig. 9 shows the distribution of Fmoc-Cys(Trt) in the PEGMA film on the silicon wafer, employing particle-based coupling (see supplementary material Video). The 3d-rendering model is based on the mass spectrometric data, collected during a ToF-SIMS depth profile, showing the trityl+−- and Si− signals.

To rule out interference of material, which could be deposited on top of the polymer film, we acquired scanning electron microscope
(SEM) images of the surface of the PEGMA film (see supplementary material Fig. S1) after particle-based immobilization and the corresponding washing steps. We thereby verified that no particle material remained on top of the sample, which might have distorted the model shown in Fig. 9.

Next, we examined the top face of the rendering-model (Fig. 9) for surface homogeneity. When transforming the ToF-SIMS measurements into a heat-map of the trityl⁺ signals distributions, the solution based (DMF) coupling reactions resulted into very uniform signals, whereas the particle-based immobilization of Fmoc-Cys(Trt)-OPip showed a leopard-like distribution of the coupled amino acid (Fig. 10). Clearly, using the particle matrix material as a medium for coupling the cysteine derivative did not result into homogeneous coverage of the synthesis surface. Thus, repetitive coupling should improve the homogeneity.

Due to its wetting properties, the viscous matrix material favors isle-like formations on the PEGMA surface (contact angle: $\theta = 41.3^\circ$ at 160°C). With respect to the high-density peptide microarray synthesis, this very same effect is highly desirable as it prevents adjacent amino acid spots from bleeding into one another. Therefore, in contrast to the SPOT-Technique, where solubilized amino acids are spotted onto the substrate, the particle-based approach allows for very high-density in situ peptide arrays.

4. Conclusion

Particle-based combinatorial coupling of amino acid building blocks results into peptide arrays of unrivelled density [13,14,18]. It is much easier to scale down the size of peptide spots by using solid materials when compared to liquids that tend to spread on the surface and to operate. Rivaling lithographic methods [10,12] suffer from another drawback when compared to the printing techniques: the 20 different amino acid building blocks have to be coupled one after another to the surface to complete a single layer of amino acids (10 x 20 = 200 coupling steps for an array of decamer peptides), leading to an intolerable amount of unwanted side reactions. In order to better understand the particle-based solid phase peptide synthesis on polymer films we scrutinized the coupling behavior of amino acid derivatives to polymer films by comparing coupling results from microparticles to solution-based amino acid coupling (as used in the SPOT-synthesis). The presented ToF-SIMS depth profiling results should lead to optimized synthesis strategies: (1) clearly, the viscous particle matrix material can only penetrate about 60 nm into the PEGMA film, which advocates for the use of thinner PEGMA layers. Less dense, and therefore, less protein resistant PEGMA layers are better suited for antibody-based bioassays, because they do not block the antibodies from accessing the surface [40]. (2) In order to achieve a homogenous coupling of the amino acid building blocks (see Fig. 10), particles with a lower glass transition temperature are preferred. These modified particle and surface parameters should result in better peptide array quality and, thereby, improve the quality of various biotechnological assays that employ high density peptide arrays. We hereby showed that ToF-SIMS requires careful control of several analytical parameters and can indeed serve as a tool to analyze a highly complex organic, chemically modified surface in 3D and in nanoscale dimensions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsusc.2015.10.223.

References


