Functionalization of Cyclo Olefin Polymer Surfaces: Comprehensive characterization and analysis of surface coatings formed by Plasma-Enhanced Chemical Vapour Deposition of (3-Aminopropyl)triethoxy-silane coatings.

Vladimir Gubala*[a], Ram Prasad Gandhiraman[a], Cedric Volcke[b], Colin Doyle[c], Connor Coyle[a,d], Bryony James[c], Stephen Daniels[a,d] and David E. Williams[e]

Received (in XXX, XXX) Xth XXXXXXXXX 200X, Accepted Xth XXXXXXXXX 200X
First published on the web Xth XXXXXXXXX 200X
DOI: 10.1039/b000000x

The surface science of bioassay devices is of great importance in the development of modern diagnostic platforms. The substrates, often disposable, must be functionalized with reactive groups to allow the active biomolecule (antibody or nucleic acid) to be immobilised at the appropriate surface density whilst maintaining its activity. More importantly, we performed a very comprehensive characterization of 3-aminopropyl-triethoxysilane films prepared under two different deposition conditions on COP slides. While the variations in the deposition conditions seemed very subtle, the use of several powerful analytical tools helped us to reveal some fundamental differences between the studied films in terms of binding capacity, swelling and adhesion. The use of such techniques is aimed to set new standards in the characterization and analysis of the substrate surface of the future diagnostic devices.

Introduction

The current trend in biomedical diagnostics, and in point-of-care applications in particular, is to use low-cost, disposable biochips that can be readily functionalized in a rapid, repeatable and controllable fashion. However, in order to fully exploit their potential, considerable research and development is still necessary, especially regarding the substrate functionalization for biomolecule immobilization[1, 2].

In general, substrates modified with organosilanes are considered to have suitable physico-chemical properties for immobilizing proteins and other biologically relevant material. Among the silanating agents, aminopropylsilanes stand out as excellent candidates for modification of silica-based material[3]. The aminopropylsilane precursor covalently couples to the surface via formation of Si-O-Si bond with the amino group extending from the surface. Numbers of papers have been published on this subject, most of them addressing the effect of different deposition conditions such as reaction temperature, incubation time, concentration, role of solvent, catalyst, adsorbed water or post- curing[4-6]. Two main techniques were well established and widely used among the surface scientists: i) when the silyl precursor is in solution and ii) in the gas phase.

The wet chemistry methods are usually the most popular, however, the film quality is sensitive to the solvent quality, given by the fact that organosilanes tend to polymerize in the presence of even trace quantities of water. Elimination of water from non-aqueous solvents together with increased amount of solvent waste makes this technique less attractive if bulk quantities of coated substrates are required.

Coatings produced by chemical vapour deposition techniques (CVD) are generally performed at elevated temperatures, which can remove the traces of water but can suffer from limitations due to dimensional tolerances and substrates that unlike silica-based material cannot withstand high temperatures.

Many of the new, state-of-the-art diagnostics platforms are based on substrates that are made of organic polymers rather than silica[7-10]. Such polymeric materials were synthesized to meet very specific criteria like excellent optical properties, good chemical resistance, cost effectivness and ease of fabrication. The latter has become of great importance as the new biochips are to be manufactured in various shapes with sophisticated microfluidics design that for instance rely on capilary forces in order to control the flow in the device[11, 12]. In some cases, the patterning of the interfacial tension of the surface is desirable. Cyclo olefin polymers (COP), are prime examples of an amorphous polymeric material that can provide a cost effective and disposable platform for biodiagnostics devices whilst maintaining the desirable properties mentioned above. For covalent attachment of biomolecules, this plastic surface needs to be functionalized. We have developed a one step process of surface functionalization, by plasma enhanced chemical vapour deposition (PECVD). This technique has number of advantages over the multistep, wet chemical methods or even CVD. It can be used to coat a large number of substrates in a single batch, it avoids direct contact with the solvent, thus reducing chemical waste and very importantly, it operates at room temperature. Also, no major limitations have been observed in terms of preparation of homogeneous coatings on curved or patterned surface and microfluidics channels[13]. It is necessary to mention that fine tuning the adhesion strength of organosilanes on certain thermoplastics such as poly(methyl methacrylate) remains still quite challenging. An ideal surface

material should have a high binding capacity but also show outstanding adhesion, stability and resistance against harsh washing and regeneration conditions.

For the preparation of homogeneous and reproducible films in bulk quantities, the coating quality and the density of the accessible active surface groups, which are the key factors permitting the reduction of the amount of sample and influencing the sensitivity of the device, require an extensive and empirical optimisation. Therefore, it is crucial to understand the relationship between the deposition conditions and both the chemical and physical characteristics of the surface.

In this paper, a comprehensive characterization of (3-aminopropyl)-triethoxysilane (APTES) films deposited by PECVD under two different conditions on COP slides known as Zeonor® is presented. One set of substrates was coated with APTES by exposing the substrates to an argon plasma containing APTES precursor for 4 minutes (A4), forming a relatively robust film of 28.15 ± 0.87 nm and water contact angle of 58°. The deposition time for the second set was 30 seconds (A30), producing thinner film of 5.12 ± 0.40 nm and water contact angle of 55°. The complexity of plasma deposited coatings requires a whole spectrum of powerful methods to analyze and characterize them. The methods used in this work are X-ray Photon Spectroscopy (XPS), Secondary Ion Mass Spectroscopy (SIMS), Atomic Force Microscopy (AFM), Dual Polarization Interferometry (DPI), Spectroscopic Ellipsometry (SE), contact angle measurement and Fluorescence Spectroscopy. These methods provide important parameters such as the chemical composition of the surface, the homogeneity of the coating, surface topography, thickness, wetability and chemical reactivity of the functional group etc. Both films were extensively characterized by qualitative and quantitative spectroscopic techniques and their stability towards hydrolysis was compared.

Experimental details

Materials

3-aminopropyltriethoxysilane (APTES), Lissamine Rhodamine B sulfonyl chloride (λexc. = 532 nm excitation and λem = 550 nm) were purchased from Sigma Aldrich, sulfosuccinimidyl-4-[2-(4,4-dimethoxytrityl)]butyrate (sSDTB) was purchased from Apollo Scientific Ltd (UK) and used without further treatment.

PECVD

The experiment was carried out in a computer controlled PECVD reactor Europlasma, model CD300 (Oudenaarde, Ghent, Belgium). An aluminium vacuum chamber, connected to a Dressler's CESAR 136 RF power source (Munsterau, Stolberg, Germany) with an operating frequency of 13.56 MHz with an automated match-box was used. The chamber details are described elsewhere[14, 15]. The COP substrates were placed at a floating electrode and the input power was fixed at 14 Watts.

The chamber was pumped down to a base pressure of 20 mTorr. Prior to the deposition, plasma cleaning and activation was carried out using argon (50sccm) + oxygen (50sccm) mix plasma (250 Watts RF power). After three minutes, the oxygen flow was closed and the RF power reduced to 14 Watts. Liqud APTES precursor was stored in a KF25 closed nipple connected through a needle valve to the chamber. As the vapor pressure of APTES is less than 10 Torr at 100°C, the APTES container was heated at 80°C and to prevent condensation of APTES in pipelines, the stainless steel supply lines from source to vacuum chamber were also heated at 80°C through a temperature controlled heating tape.

XPS

The XPS data were collected on a Kratos Axis UltraDLD equipped with a hemispherical electron energy analyser. Spectra were excited using monochromatic Al Kα X-rays (1486.69 eV) with the X-ray source operating at 100W. This instrument illuminates a large area on the surface and then using hybrid magnetic and electrostatic lenses collects photoelectrons from a desired location on the surface. In this case the analysis area was a 220 by 220 micron spot. The measurements were carried out in a normal emission geometry. A charge neutralisation system was used to alleviate sample charge buildup, resulting in a shift of approximately 3eV to lower binding energy. Survey scans were collected with 160eV pass energy, whilst core level scans were collected with a pass energy of 20eV. The analysis chamber was at pressures in the 10-9 torr range throughout the data collection.

Data analysis was performed using CasaXPS (www.casaXPS.com). Shirley backgrounds were used in the peak fitting. Quantification of survey scans utilised relative sensitivity factors supplied with the instrument. Core level data were fitted using Gaussian-Lorentzian peaks (30% Lorentzian). The binding energy scale was corrected for the neutraliser shift by using the C 1s signal from saturated hydrocarbon at 285.0 eV as an internal standard. The elements present in the coating C, N, O, Si were detected using the XPS survey scan. High resolution scans of individual core levels showed the various bonding states.

Surface SIMS

Secondary Ion Mass spectrometry (SIMS) is, in spite of its destructive nature, one of the most appealing methods thanks to its ability to provide specific chemical information about the layers through the analysis of their fragmentation obtained by bombardment with a perpendicular focused ion beam. The secondary ion mass spectroscopic studies were carried out using a quadrupole apparatus MiniSIMS developed by Millbrook Instruments Ltd. It incorporates a raster scanned gallium liquid metal ion gun for the primary beam and low-energy optics for secondary ion extraction into a 300 Da quadrupole. Ga+ ions (6 keV) were focussed perpendicularly to the substrate. To mitigate charging of the electrically insulating zeonor substrate an electron gun charge neutralisation was used for all the measurements. The operating pressure was 3.1×10^{-7} mbar and the chemical imaging was performed in a broad beam mode.

Dual Polarization Interferometry

Dual polarization interferometry, an optical sensing technique, was carried out using a Farfield AnaLight® instrument. This measurement required special substrates, not Zeonor, but offered some significant advantages for probing in-situ the interactions of the plasma-formed coatings with aqueous media. For this measurement, the coatings had to be deposited onto chips whose surface was an air-formed silica. The Silicon oxynitride AnaChipTM consists of two optical waveguides, that confine light in defined boundaries, stacked one on top of the other. The substrate is a silicon wafer and the waveguides are silicon dioxide doped with silicon nitride. A collimated light beam from a helium-neon laser (wavelength 632.8 nm) is first passed through a ferro electric liquid crystal ½ wave plate to create plane polarised light. The instrument measures the phase retardation of the guided light caused by interaction of the evanescent field with coatings on the top waveguide, by measurement of an interference pattern developed between the sample beam and the un-retarded reference beam passing through the bottom waveguide. It utilises the different evanescent field penetration depth of light polarised perpendicular- and parallel-to the waveguide surface to derive thickness and refractive index information about the surface coatings. DPI has been verified using standard protein systems and has been demonstrated in the successful monitoring of biochemical interactions, e.g., protein adsorption[16, 17] and lipid membrane formation[18, 19]. The specific purpose of these measurements was to investigate the structural changes caused to the film by washing with surfactant solutions. The structural changes taking place with the addition of a surfactant were investigated by monitoring the refractive index, thickness and mass variations following exposure to PBS Tween. The temperature was kept at 20°C.

Quantification of amine groups using SulfoSDTB

The samples were incubated for 30 minutes at room temperature in a freshly prepared solution of sulfosuccinimidyl-4-[2-(4,4-dimethoxytrityl)]butyrate (sSDTB) (0.1 mM at pH = 8.0). After incubation, all substrates were thoroughly rinsed with water and then treated with 37.5 % perchloric acid to allow the formation of 4,4'-dimethoxytrityl cation from the substrates. Since the reaction between the amines and the sSDTB proceeds with 1:1 stoichiometric ratio, the concentration of the released cation measured bu UV/vis spectrophotometer at 498 nm was used to quantify the amine group density per cm^2.

Atomic force microscopy imaging (AFM)

AFM examinations are performed with a commercial microscope (Dimension 3100 controlled by a Nanoscope IIIa controller, Digital Instruments, Santa-Barbara – CA, USA), in the Tapping Mode™, using standard silicon cantilevers (BudgetSensors®, Innovative Solutions Bulgaria Ltd, Bulgaria) with a 7 nm radius of curvature and a 42 $N.m^{-1}$ spring constant (nominal values). Topographic images were recorded at a scanning rate of 1-2 Hz, and a resonance frequency of about 300 kHz (nominal value). The background slope was resolved using first order polynomial function. No further filtering was performed. The surface roughness of APTES coated COP substrates was evaluated over 3 images (2 µm x 2 µm) and the standard deviation calculated. The root-mean-square roughness (Rrms) was calculated from height deviations taken from the mean plane.

Fluorescence

Fluorophore attachment to amino-functionalized Zeonor substrates was achieved by immersing the samples for 1 hour in a 0.23 mM lissamine rhodamine B sulfonyl chloride in acetonitrile. 100 µl triethylamine were added to 100 ml of the lissamine solution. Samples were then rinsed with distilled water and sonicated for 5 minutes in a 0.1 % w/v sodium dodecyl sulfate (SDS) solution, followed by a rinse with distilled water and dried under a stream of nitrogen. The fluorescence intensity after lissamine attachment was measured using a GMS 418 scanner (Genetic Microsystems, Affymetrix).

Results and discussion

In the PECVD process, the input radio frequency (RF) current / voltage is supplied to the powered electrode with respect to the grounded chamber for creating the plasma. Charged species (free electrons and ions) present in the chamber are accelerated by the electric field and collide with molecules of the source gases. In this way, the source gas molecules are excited to higher energy states, primarily by inelastic collisions with the energetic electrons, and dissociate into a variety of radicals, ions, atoms and more electrons. Radicals and atoms, generated in the plasma, travel to the growing film surface through a gas phase diffusion process. They are then adsorbed onto the surface and form chemical bonds at favourable sites to create an amorphous network. We have recently proposed a reasonable mechanism on the formation of a siloxane-silicone network in APTES deposition by PECVD in the presence of traces of water[20]. We reason that the siloxane-silicone network formation could be initiated by fragmentation of APTES into aminosiloxane radicals that condense and polymerise on the surface. Further fragmentation of these radicals (e.g. by applying higher input RF power or deposition time exceeding 8 minutes) does not lead into formation of a stable, adherent, amine-reactive network on COP[21]. Similarly, such stable films were not formed in the absence of the siloxane functionality. The siloxane functionality seems to be essential both for insertion into C-C bonds of the COP substrate and for building a stable, polymerized network.

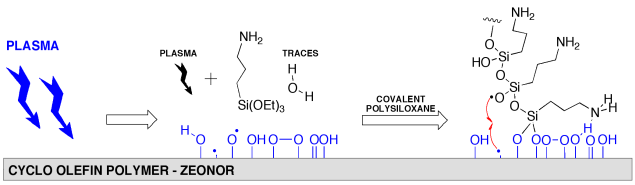

Fig. 1 Proposed reaction mechanism of APTES on oxidized Zeonor surface by PECVD. Shorter deposition times (30 seconds) lead into formation of thinner films when compared to longer reaction times (4 minutes).

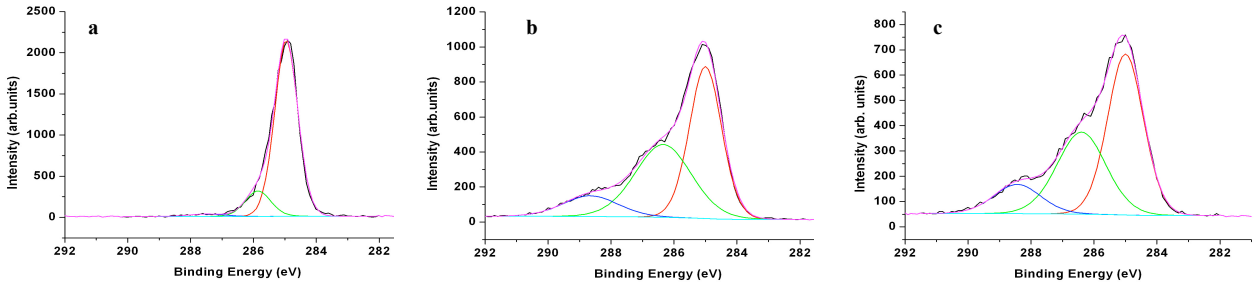

Fig. 2 High resolution C 1s core level photoemission spectra of (a) untreated COP surface, (b) APTES 30sec (c) APTES 4 min coatings on COP substrates using monochromatic Al Kα monochromatic X-rays (1486.69 eV)

The COP substrate was in one case exposed to the fragmented aminosiloxane radicals for 30 seconds, forming a relatively thin polysiloxane layer of 5.12 ± 0.40 nm. In the other case, the reaction time was extended to 4 minutes, which lead into a formation of more robust, 28.15 ± 0.87 nm thick film. While both coatings contain the desired amines, their surface and bulk characteristics vary and are in discussed below.

Qualitative characterization by X-ray Photoelectron Spectroscopy (XPS)

Firstly, the elements present in the coatings (C, O, Si and N) were identified by XPS survey spectra (data not shown). For further analysis, high-resolution spectra were recorded from individual peaks. Qualitatively, the C 1s spectra for all films (Figure 2) look reasonably similar with one saturated hydrocarbon peak (285.0 eV) and two additional peaks of higher binding energy at 286.4 eV (~+1.4) and 288.4 eV (~+3.4). The +1.4 peak could be assign to C-N bond as well as C-O-C, -C-OH, C=N, and C-O-Si, whilst the higher binding energy peak (~+3.4) is characteristic for C≡N, COOH and $CONH_2$. The assignements were done based on number of communications that deal with the changes in chemistry and morphology of COP substrates either just after plasma oxidation[22-26] or the subsequent depositions of alkylsiloxanes, such as APTES. In light of these reports we rationalize that it is not very likely that the polymerized siloxane films contain C-O-C or -C-OH bonds. Therefore, the peak at 286.4 eV is best attributed to C-N bonding, corresponding to the terminal $–CH_2-NH_2$ functionality.

Table 1 Elemental analysis by XPS

Sample	C 1s		At % in the sample
	Binding Energy (eV)	Attribution	
Zeonor®	284.6 (84.0%) 285.5 (13.9%) 287.1 (2.1%)	C-C Vibr. component of C-C. Surface oxide	C 1s – 98.5 O 1s – 1.1 Si 2p – 0.4
A30	284.7 (48.3%) 286.0 (40.4%) 288.4 (11.3%)	C-C C-N $CO-NH_2$	C 1s – 57.2 N 1s – 4.4 O 1s – 28.3 Si – 10.2
A4	285.0 (51.7%) **286.4 (36.0%)** 288.4 (12.3%)	C-C **C-N** $CO-NH_2$	C 1s – 49.0 **N 1s – 5.8** O 1s – 30.7 Si 2p – 14.5

Similarly, it is less probable for the coating to contain C triple bond N, so the peak at 288.4 eV was assigned to C1s of either -COOH or $–CONH_2$ group. The deconvoluted C 1s spectrum of the plain COP substrate (Figure 1a) shows an additional peak at 285.5 eV along with the major C-C peak[27].

Quantitatively, as seen from the C 1s spectra, the relative amount of C-N bonding was higher in A30 coating (40.4 %) than the A4 coating (36%), indicating a higher quantity of amino groups in the former (Table 1).

Chemical imaging and depth profiling using surface Secondary Ion Mass Spectrometry (SIMS)

SIMS is one of the most sensitive surface analysis techniques to analyze the composition of solid surfaces and thin films by sputtering the surface of the studied sample with a focused primary ion beam and collecting and analyzing ejected secondary ions. The secondary ion chemical imaging of the both A30 and A4 substrates was taken for m/z = 26 in negative ion SIMS mode (–C-N fragment).

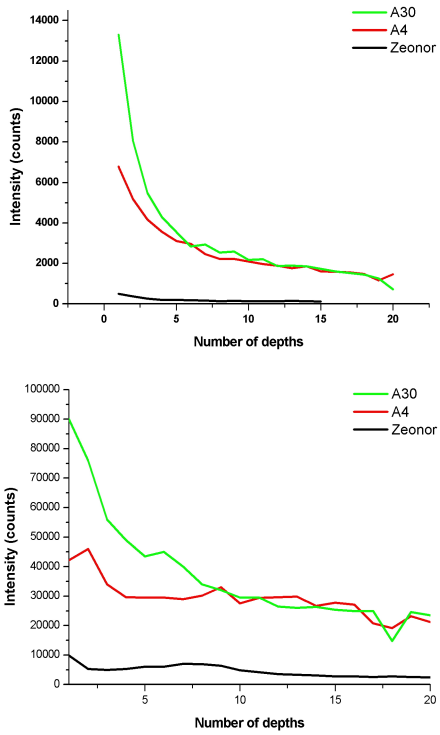

Fig. 3 Depth profile analysis of (a) –CH2N+ fragment at m/z = 28 with positive SIMS and (b) –CN- fragment at m/z = 26 with negative SIMS

The depth profile measurements, depicted on figure 3, were carried out for $–CH_2N^+$ at m/z = 28 in positive SIMS and $–CN^-$ at m/z = 26 in negative SIMS. A30, A4 slides and also plain Zeonor as negative control were fragmented several times (on the X axis of Figure 3 marked as 'Number of depths') by bombardment with a perpendicular focused ion beam and the signal intensity of ejected secondary ions was recorded. While no fragments were detected from plain Zeonor, the signal intensities of both the CH_2N^+ and $–CN^-$ ions were significantly high for both APTES coatings. As seen on figure 3, the number of ion counts for the A30 after the initial etching were much higher then those of the A4 films, confirming higher density of amines on the A30 film. The signal reached plateu as soon as the coatings were etched to such depth, where no more fragments were ejected.

Coating thickness and swelling in contact with water: Dual Polarisation interferometry and Atomic Force Microscopy

The molecular scale and composition of both coatings were further probed by DPI. It is a technique based on a dual waveguide interferometry. By defining the waveguide structure with alternate polarizations both the refractive index and the thickness of adsorbed layers at the substrate (solid) – liquid interface were determined.

Information about the mass and the density of the adsorbed A30 and A4 films were derived, based on which the amount of amino groups for both A30 and A4 samples was calculated, assuming that the silane does not fragment completely but retains its aminosiloxane molecular identity. Therefore, from the mass of the film per cm^2 and the molar mass of the monomer, the number of moles of $–NH_2$ in the film per cm^2 of geometric area can be calculated. DPI allowed monitoring the layer composition of each slide before and after a treatment with 1% w/v solution of PBS Tween (PBST) to probe the film adhesion and the structural changes upon washing with a detergent. DPI also revealed that the A30 coatings undergo some swelling when in contact with PBS buffer, which is reflected in the increased thickness of the layer from 5.12 to 6.39 nm. PBST treatment leaves A30 film virtually unaffected, keeping the APTES mass constant at 3.80 ng/cm^2. This is contrary to A4 slide that shows very little swelling when treated with PBS buffer (thickness increase from 29.95 nm to 30.03 nm). Also the mass of APTES on A4 film drops from 17.7 ng/cm^2 to 15.1 ng/cm^2 after washing with Tween.

Table 2 Thickness, mass and the number of amines on A30 and A4 slides before and after treatment with PBS Tween, measured by DPI.

	untreated			treated		
	Thickness [nm]	Mass [ng/mm^2]	# of $-NH_2$ [per cm^2]	Thickness [nm]	Mass [ng/mm^2]	# of $-NH_2$ [per cm^2]
A4	29.95	17.7	77.77×10^{14}	30.03	15.1	66.43×10^{14}
A30	5.12	3.80	16.69×10^{14}	6.39	3.80	16.71×10^{14}

AFM imaging was employed to review the potential variations in coating structure upon changing environment. TM – AFM topographical images of the samples were measured on air, in deionised water and also PBS buffer. The results are presented in figure 4.

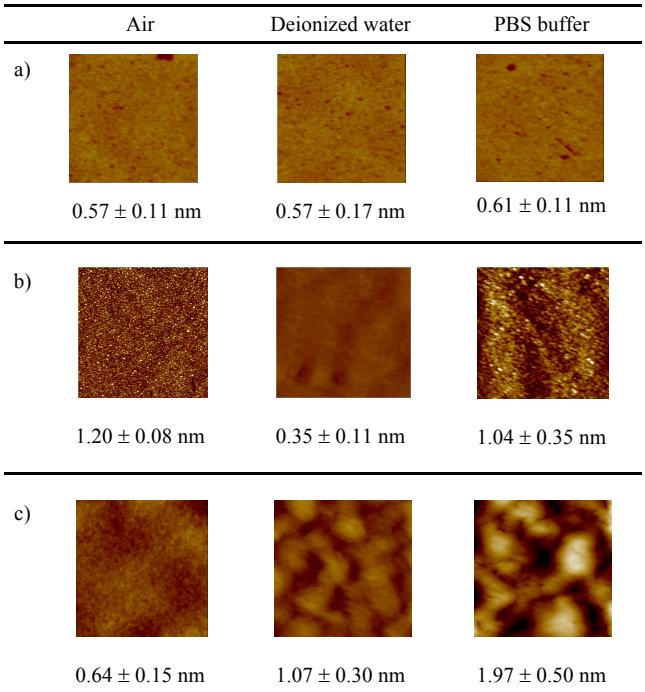

Fig. 4 The effect of various environments on surface roughness. AFM images of plain Zeonor (a), A30 (b) and A4 (c) slides measured in air, deionized water and PBS buffer.

A dry A30 sample exhibits higher roughness than dry A4 slide, however, dramatic change is observed when both samples are immersed in deionized water and PBS buffer. A4 is a thicker film and when dry, it shows the roughness resulting from a longer exposure to the silane – i.e. flat, polymerized film. The high amount of amino groups in the bulk causes that upon contact with molecules of water, the surface roughens up. This is on the contrary to the wet A30 slides that undergo conformational changes most likely triggered by swelling, which results in improvement of the surface roughness, shown by RMS index decreasing from 1.20 to 0.35 nm. Deposition of salts from PBS buffer brings the RMS value back to 1.04, nonetheless, it is still lower than that of A4 sample. These results are in concert with the data observed by DPI.

Reactive amine fraction, and its stability against surfactant treatment

The results were corroborated with colorimetric measurement with sSDTB[28]. This simple experiment was performed to reveal the number of reactive amino groups on both coatings before and after treatment with PBS Tween. The data are summarized in Table 3. As determined by DPI, the total number of $–NH_2$ groups per cm^2 present in the ~30 nm A4 film was nearly five times higher than the one in the thinner, ~5 nm A30 film. However, the chemically-available, reactive amine content of the A30 coatings was nearly double that of the A4. Apparently, only less than 10 % of the total amount of the amino groups was available for the reaction with sSDTB in case of A4 film, while more than 40 % of the

amines on the thinner A30 slides were still accessible for the same reaction.

Table 3 Average number of amine groups on A30 and A4 slides determined by Dual Polarization Interferometry and colorimetric reaction with sSDTB. The numbers are per cm^2.

	untreated		treated	
	A4	A30	A4	A30
# of all -NH_2 by DPI [x 10^{14}]	77.77	16.69	66.43	16.71
# of free -NH_2 by sSDTB [x 10^{14}]	3.99 ± 0.44	7.01 ± 0.28	4.82 ± 0.81	6.20 ± 0.06
% of bulk NH_2	94.9	58.0	92.7	62.9

Adhesion study by extensive washing of fluorophore Lissamine Rhodamine

A routine manipulation with substrates in immunoassays involves extensive washing with aqueous solutions containing detergents. Good adhesion and stability of coatings against harsh washing and hydrolysis are therefore necessary to ensure reproducibility and precision of the device. Lissamine Rhodamine sulfonyl chloride was covalently attached to both A30 and A4 substrates and the slides were subject to extensive washing with water and also with PBST. The amount of fluorophore washed off at every rinsing step was determined by means of fluorescence.

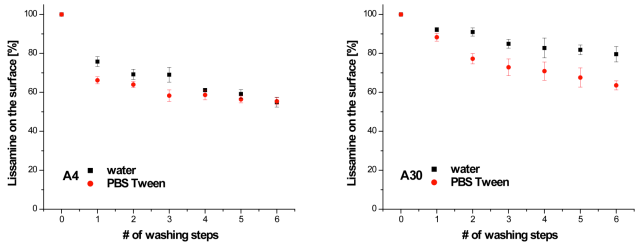

Fig. 5 Percentage of Lissamine Rhodamine that remained attached on the surface after each one of the six rinsing cycles with either water or PBS Tween.

Both silane films showed reasonable adhesion strength and resistance against washing with either water or PBS Tween. Nonetheless, the A30 film performed better than A4, after six washing steps in water, ~80% of the Lissamine still remained attached on the surface. The more aggressive detergent, PBS Tween, was more efficient, removing nearly 30% of the loosely bound fluorophore, leaving ~70% of Lissamine Rhodamine covalently bound to the sample.

Conclusions

In this article, we have demonstrated how subtle difference in the PECVD parameters can influence not only the thickness of the deposited nanolayer but also its bulk properties. More importantly, careful examination of the film characteristics revealed significant differences in binding capacity, swelling and adhesion between the two measured samples. Various surface characterization techniques, many of them readily accessible to surface scientists have been employed and aimed to understand and further control the fabrication of delicate nanostructures by PECVD on thermoplastic substrates such as COP. Amine functional coating deposited for 30 seconds showed by all means superior properties when compared with its 4 minutes counter part demonstrating that the growing film that was exposed to plasma for shorter duration results in higher number of the active binding sites. The faculty of being able to prepare nanometer size layers of organic silanes in bulk quantities with high density of binding groups, excellent adhesion and wetting make us very optimistic on the possibility of sucessful and widespread application of PECVD in surface functionalization of next generation biosensor device platforms.

Notes and references

[a] *Biomedical Diagnostics Institute (BDI) Dublin City University, Collins Avenue, Glasnevin, Dublin 9, Ireland. Fax: +353 1 700 6558; Tel: +353 1 700 6558; E-mail: vladimir.gubala@dcu.ie*

[b] *Research Centre in Physics of Matter and Radiation (PMR), University of Namur (FUNDP), 61, rue de Bruxelles, B-5000 Namur, Belgium. Fax: XX XXXX XXXX; Tel: +3281725430; E-mail:* cedric.volcke@fundp.ac.be

[c] *Research Centre for Surface and Materials Science, Department of Chemical and Materials Engineering,University of Auckland, Private Bag 92019, Auckland 1142, New Zealand. Fax: +64 9 373 7463; Tel: +64 9 373 7599; E-mail:* b.james@auckland.ac.nz, c.doyle@auckland.ac.nz

[d] *National Centre for Plasma Science and Technology, Dublin City University,Collins Avenue, Glasnevin, Dublin 9, Ireland. Fax: +353 1 700 8021; Tel: +353 86 807 8413; E-mail:* stephen.daniels@dcu.ie

[e] *MacDiarmid Institute for Advanced Materials and Nanotechnology, Department of Chemistry,University of Auckland, Private Bag 92019, Auckland 1142, New Zealand. Fax: +64 4 463 5237; Tel: +64 9 373 7599; E-mail:* david.williams@auckland.ac.nz

1. M. Vareiro, I. Tranchant, S. Maplin, K. Zak, M. M. Gani, C. J. Slevin, H. C. Hailes, A. B. Tabor, A. T. A. Jenkins and D. E. Williams, *Anal. Biochem*, 2008, **377**, 243.
2. H. Xu, J. R. Lu and D. E. Williams, *J. Phys. Chem. B*, 2006, **110**, 1907.
3. P. VanDerVoort and E. F. Vansant, *Journal of Liquid Chromatography & Related Technologies*, 1996, **19**, 2723-2752.
4. P. Silberzan, L. Leger, D. Ausserre and J. J. Benattar, *Langmuir*, 1991, **7**, 1647-1651.
5. S. A. Kanan, W. T. Y. Tze and C. P. Tripp, *Langmuir*, 2002, **18**, 6623-6627.
6. J. Buijs and D. D. White, *Colloids Surf. B*, 1997, **8**, 239.
7. M. Yamazaki, *Journal of Molecular Catalysis a-Chemical*, 2004, **213**, 81-87.
8. K. Obuchi, M. Komatsu and K. Minami, *Optical Manufacturing and Testing Vii*, 2007, **6671**, U399-U407 560.
9. W. D. Niles and P. J. Coassin, *Assay and Drug Development Technologies*, 2008, **6**, 577-590.
10. L. Yi, X. D. Wang and Y. Fan, *Journal of Materials Processing Technology*, 2008, **208**, 63-69.

11. D. Kurzbuch, J. Bakker, J. Melin, C. Jonsson, T. Ruckstuhl and B. D. MacCraith, *Sensors and Actuators B-Chemical*, 2009, **137**, 1-6.
12. C. Jonsson, M. Aronsson, G. Rundstrom, C. Pettersson, I. Mendel-Hartvig, J. Bakker, E. Martinsson, B. Liedberg, B. MacCraith, O. Ohman and J. Melin, *Lab on a Chip*, 2008, **8**, 1191-1197.
13. R. P. Gandhiraman, S. K. Karkari, S. Daniels and B. MacCraith, *Surface and Coatings Technology*, 2009, **203**, 3521-3526.
14. G. R. Prasad, S. Daniels and D. C. Cameron, *Plasma Processes and polymers* 2007, **4**, 369-373.
15. G. R. Prasad, S. Daniels, D. C. Cameron, B. P. McNamara, E. Tully and R. O'Kennedy, *Surface & Coatings Technology*, 2005, **200**, 1031-1035.
16. G. H. Cross, A. A. Reeves, S. Brand, J. F. Popplewell, L. L. Peel, M. J. Swann and N. J. Freeman, *Biosensors & Bioelectronics*, 2003, **19**, 383-390.
17. M. J. Swann, L. L. Peel, S. Carrington and N. J. Freeman, *Analytical Biochemistry*, 2004, **329**, 190-198.
18. J. F. Popplewell, M. J. Swann, N. J. Freeman, C. McDonnell and R. C. Ford, *Biochimica Et Biophysica Acta-Biomembranes*, 2007, **1768**, 13-20.
19. C. J. Terry, J. F. Popplewell, M. J. Swann, N. J. Freeman and D. G. Fernig, *Biosensors & Bioelectronics*, 2006, **22**, 627-632.
20. R. P. Gandhiraman, C. Volcke, V. Gubala, C. Doyle, L. Basabe-Desmonts, C. Dotzler, M. F. Toney, M. Iacono, R. I. Nooney, S. Daniels, B. James and D. E. Williams, *J. Mater. Chem.*, 2010, **in press**.
21. C. Volcke, R. P. Gandhiraman, V. Gubala, J. Raj, T. Cummins, G. Fonder, R. I. Nooney, Z. Mekhalif, G. Herzog, S. Daniels, D. W. M. Arrigan, A. A. Cafolla and D. E. Williams, *Biosensors & Bioelectronics*, 2010, **25**, 1875-1880.
22. H. Shinohara, J. Mizuno and S. Shoji, *Ieej Transactions on Electrical and Electronic Engineering*, 2007, **2**, 301-306.
23. Y. J. Kim, Y. Taniguchi, K. Murase, Y. Taguchi and H. Sugimura, *Applied Surface Science*, 2009, **255**, 3648-3654.
24. S. Okuji, M. Sekiya, M. Nakabayash, H. Endo, N. Sakud and K. Nagai, *Nuclear Instruments & Methods in Physics Research Section B-Beam Interactions with Materials and Atoms*, 2006, **242**, 353-356.
25. S. J. Hwang, M. C. Tseng, J. R. Shu and H. H. Yu, *Surface & Coatings Technology*, 2008, **202**, 3669-3674.
26. T. M. Wu and C. W. Wu, *Journal of Polymer Science Part B-Polymer Physics*, 2005, **43**, 2745-2753.
27. G. Beamson and D. Briggs, *High resolution XPS of organic polymers: the Scienta ESCA300 database*, Chichester, New York, 1992.
28. S. Fiorilli, P. Rivolo, E. Descrovi, C. Ricciardi, L. Pasquardini, L. Lunelli, L. Vanzetti, C. Pederzolli, B. Onida and E. Garrone, *Journal of Colloid and Interface Science*, 2008, **321**, 235-241.