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Analyst

Cite this: *Analyst*, 2011, **136**, 3496

www.rsc.org/analyst

PAPER

Selective functionalisation of PDMS-based photonic lab on a chip for biosensing†‡

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Received 24th November 2010, Accepted 6th January 2011
DOI: 10.1039/c0an00941e

Published on 18 February 2011 on http://pubs.rsc.org | doi:10.1039/C0AN00941E
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A comparative study of different approaches for the selective immobilisation of biomolecules on the surface of poly(dimethylsiloxane) (PDMS) is reported. The motivation of this work is to set a robust and reliable protocol for the easy implementation of a biosensor device in a PDMS-based photonic lab-on-a-chip (PhLoC). A hollow prism configuration, previously reported for the colorimetric detection of analytes was chosen for this study. Here, the inner walls of the hollow prism were initially modified by direct adsorption of either polyethylene glycol (PEG) or polyvinyl alcohol (PVA) linear polymers as well as by carrying out a light chemical oxidation step. All these processes introduced hydroxyl groups on the PDMS surface to a different extent. The hydroxyl groups were further silanised using a silane containing an aldehyde end-group. The interaction between this group and a primary amine moiety enabled the selective covalent attachment of a biomolecule on the PDMS surface. A thorough structural characterisation of the resulting modified-PDMS substrates was carried out by contact angle measurements, X-ray photoelectron spectroscopic (XPS) analysis and atomic force microscopy (AFM) imaging. Using horseradish peroxidase as a model recognition element, different biosensor approaches based on each modification process were developed for the detection of hydrogen peroxide target analyte in a concentration range from 0.1 μM to 100 μM. The analytical performance was similar in all cases, a linear concentration range between 0.1 μM and 24.2 μM, a sensitivity of 0.02 a.u. μM^{-1} and a limit of detection around 0.1 μM were achieved. However, important differences were observed in the reproducibility of the devices as well as in their operational stability, which was studied over a period of up to two months. Considering all these studies, the PVA-modified approach appeared to be the most suitable one for the simple fabrication of a biosensor device integrated in a PDMS PhLoC.

Introduction

Microfluidics is the science devoted to the study of fluids in channels with a size that varies from the units to the hundreds of micrometres. Making use of microfluidics it is possible to fabricate Lab-on-chip (LoC) systems, miniaturising diverse functionalities that typically require a whole (bio)chemical laboratory. These systems provide several advantages compared to the standard ones, such as high sensitivity, faster analysis, decrease in sample, reagent and waste volume, automatisation and standardisation of processes.[1,2]

The huge impact of the LoC concept cannot be understood without the development of polymeric materials. Indeed, polycarbonate (PC), poly (methyl methacrylate) (PMMA) and poly(dimethylsiloxane) (PDMS) have been widely applied for the fabrication of LoC, thereby providing high versatility and reduction of both time and cost of the fabrication process. By using PDMS, microsystems can rapidly be fabricated by cast moulding with resolutions down to 0.1 μm (ref. 3) and in turn easily sealed to a variety of different substrates.[4] It is a cheap material that polymerises at low temperatures,[5] it is optically transparent in a very wide wavelength range (from UV to NIR) and as such is compatible with many optical detection methods. Its non-permeability to water, non-toxicity and permeability to gases makes it fully compatible with biological studies. It is flexible and elastic, with a Young's modulus of 2.5 MPa when it is fabricated with a 10 : 1 ratio of base : curing agent.[6] This elasticity enables its easy releasing from the master during the fabrication process and it also facilitates the mechanical pumping of fluids inside the microchannels.[5,7] Among the different transduction modes applied in LoC technology, those systems that use

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† This article is part of a themed issue on Emerging Investigators.
‡ Electronic supplementary information (ESI) available. See DOI: 10.1039/c0an00941e

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light as interrogation mechanism (usually called photonic LoCs or, PhLoC) exhibit some advantages. These include immunity to electromagnetic interferences and/or multiplexed detection in a single system.[8] PhLoCs have already successfully been applied to the detection and quantification of different targets such as cells,[9] DNA[10] or glucose.[11] In addition, PhLoCs can also take advantage of the optical properties of PDMS, thus enabling the fabrication of low-cost systems with the potential of being highly sensitive, and easy-to-integrate with other microoptic and microfluidic elements.

One important drawback of PDMS lies in its ability to non-specifically adsorb biomolecules and other macromolecules, which hampers its application for chemical sensing. In this context, its surface can be easily tailored in order to avoid such undesirable process or, by contrast, to selectively immobilise different molecules.[12] The application of PDMS-based microsystems to (bio)chemical analysis and other disciplines requires carrying out surface modification processes, both chemical and physical, aiming at, for instance, decreasing biomolecular adsorption, as pointed out above, or increasing the hydrophilic/hydrophobic character of the surface. Moreover, other processes are directed to attach a biologically active molecule that alters the lubricity of the polymer surface[13] or provides the material with the capacity to give a selective answer to a specific target analyte.[14] Surface modification techniques can be divided into two groups: physical adsorption and covalent modification. The first one has been widely used due to its great simplicity but the resulting surfaces present thermal, mechanical and solvolytic instability because the interactions between the adsorbed material and the surface are weak. Covalent modification can overcome these drawbacks and give rise to a more robust modification of the surface.[15] In this context, PDMS surfaces have been treated with oxygen plasma[16] or UV/ozone,[17] in order to make the surface hydrophilic by replacing the surface methyl groups, bound to the Si on the PDMS structure, by silanol groups (Si–OH). These new groups are prone to chemically interact with different functional groups, thus enabling the selective modification of the PDMS surface. However, it should be taken into account that the properties of these silanol groups are dynamic in the sense that the surface progressively recovers its hydrophobicity. Keeping in mind this drawback, the fact that these processes required special instrumentation and cannot be applied in microfluidic channels that were embedded in PDMS matrices,[18] alternative processes for the selective modification of PDMS surfaces, which were easy to implement, are required.

In this work, a study of different liquid-based surface chemical biofunctionalisation methods was carried out using a PDMS PhLoC consisting of a hollow Abbe prism transducer configuration (Fig. 1).[19] On one hand, physical adsorption of two different polymers containing hydroxyl groups, such as polyethylene glycol (PEG) or polyvinyl alcohol (PVA) enabled the further silanisation of the surface for the introduction of chemical functional groups and the eventual covalent immobilisation of the protein receptor. Both polymers were previously applied to the modification of PDMS in order to avoid the non-specific binding of proteins.[20,21] On the other hand, a covalent modification approach was tested based on the chemical oxidation of the PDMS surface, thus generating silanol groups onto which a silanisation process and further immobilisation of the protein receptor were carried out, as above. A thorough structural and analytical characterisation of the resulting modified PDMS surfaces was carried out. These methods could be performed in chemical and biological laboratories and applied to PDMS microfluidic systems without the need for special instrumentation.

Materials and methods

Chemicals and materials

The EPON SU-825 photoresist and the propylene glycol methyl ether acetate (PGMEA) were purchased from MicroChem Corporation (Newton, MA, USA). The PDMS Sylgard 184 elastomer kit was bought from Dow Corning (Midland, MI, USA) and used according to the datasheet.

99% PVA, PEG (molecular weight: 12 000 g), 30% (v/v) H_2O_2, 99% triethylamine (TEA), HRP type VI, 99% 2,2′azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), sodium cyanoborohydride ($NaBH_3CN$) and Tween 20 were from Sigma-Aldrich Co. (St Louis, MO 63103, USA). 90% 11-triethoxysilyl undecanal (TESU) was from ABCR GmbH & Co. KG (76187 Karlsruhe, Germany). All other chemicals were of analytical grade.

Fabrication of the microsystem

The PhLoC was fabricated by cast moulding, following a previously reported protocol.[22] First, a master was fabricated with EPON SU-8 25 negative photoresist. A spin coating process of this photoresist was twice carried out with the aim of reaching a height of 250 μm. The resist was dried at 95 °C for 3 hours and then a standard UV-photolithographic process was carried out, followed by a baking step for 20 min at 95 °C and a consecutive developing step in PGMEA. The final step was a hard bake (HB) at 120 °C for 2 hours in an inert atmosphere so as to harden the master. Then, PDMS was used for the replication of the master. Pre-polymer was prepared by mixing the curing agent and the base elastomer in a 1 : 10 (v/v) proportion and degasifying it in a vacuum chamber. After pouring the pre-polymer on the master, the PDMS was cured on a hot plate at 80 °C for 20 min. The PDMS was then peeled off from the mould and sealed over

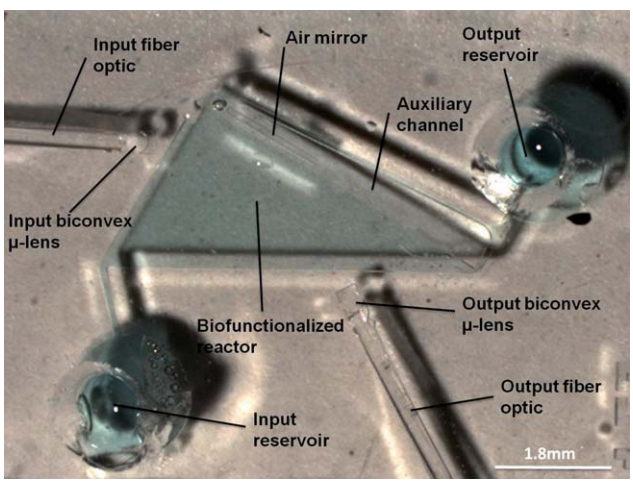

Fig. 1 Picture of the PDMS PhLoC microfluidic system.

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a flat soda-lime glass substrate. For the sealing, both PDMS and glass surfaces received a plasma treatment.[23] That is, they were placed in a barrel etcher (Surface Technology Systems, Newport, UK) and exposed to oxygen plasma (75 sccm O_2, 85 W, 30 seconds). Immediately after the treatment they were put in contact and kept at 70 °C for 30 min, thus irreversibly sealing the fluidic system. Finally, the fluidic ports were opened the final volume of the PhLoC is 1.6 μL.

Modification and characterization of the PDMS surfaces

The three selected approaches for the PDMS surface modification are based on the introduction of hydroxyl (–OH) groups. Fig. 2 shows a scheme of the different steps required for each functionalization process. First, the PDMS surfaces were cleaned with ethanol and deionised water (DI H_2O). For the modification shown in Fig. 2A, a 1 mg ml^{-1} PEG solution in DI H_2O was pumped inside the system and left to react for 1 hour at room temperature (RT). For the modification in Fig. 2B, the system was filled with a 1 mg ml^{-1} PVA solution in DI H_2O and left to react for 1 hour at room temperature (RT). Fig. 2C shows the direct introduction of –OH groups on the PDMS surface, using an acidic solution containing DI H_2O, 37% HCl and 30% H_2O_2 in a 5 : 1 : 1 (v/v/v) ratio.[18] After each of these steps, the surfaces were rinsed with DI H_2O and dried under a N_2 stream. Next, a silanisation process was carried out by incubating the modified PDMS systems in a 99.5% ethanol solution containing 2% TESU (2%) and 2% TEA for 1 hour at RT. Then the surfaces were thoroughly rinsed with 99.5% ethanol and dried at 80 °C for 2 hours. The TEA induces a highly nucleophilic oxygen in the –OH group that readily interacts with a silane[24] having its ethoxy groups previously hydrolysed. In this way, the silane covalently bound to the surface. All these modifications were also applied to flat PDMS surfaces having an area of 1 cm^2 to facilitate the structural characterisation of the resulting modified material by the different techniques described below.

Contact angle measurements were carried out with the sessile drop method, using a Krüss Easydrop contact angle meter and DS1 analysis software (Krüss GmbH, Hamburg, Germany). A drop of water was deposited on the modified PDMS surface and the angle formed between the liquid and the solid surface was measured. XPS analysis was carried out at the facilities of the Instituto de Nanociencia de Aragon (Spain) on an Axis Ultra-DLD spectrometer (Kratos Analytical Ltd, Manchester, England), using a monochromatised Al Kα source (1486.6 eV). Signals were deconvoluted with the software provided by the manufacturer, using a weighted sum of Lorentzian and Gaussian component curves after background subtraction. The binding energies were referenced to the internal standard C1s (284.9 eV). Atomic force microscopy topographic and phase images of the modified surfaces were taken with a Veeco Nanoscope Dimension 3100 (Veeco, Plainview, NY, USA), working in tapping mode.

Fabrication of the biosensor approach

The analytical performance of the different modification protocols was tested by developing a biosensor for the detection of H_2O_2 based on the use of HRP as a model recognition element. The resulting aldehyde-modified PDMS surfaces bound HRP through the amine groups of its lysine residues, by forming a Schiff base that is further reduced to a stable secondary amine with sodium cyanoborohydride.[25] HRP catalyzes the reduction of H_2O_2 in the presence of colourless 2,2′azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) charge transfer mediator, which is concomitantly oxidized to the green-coloured $ABTS^+$ radical cation (Fig. S1, ESI‡).[26] This redox species shows an absorption peak at 420 nm and two secondary peaks at 650 nm and 720 nm, the first one being chosen for the absorbance detection of this enzymatic reaction.

The overall procedure is described in detail below. PhLoC was filled with a solution containing 1 mg ml^{-1} HRP type VI and 5 mM sodium cyanoborohydride in 0.05 M carbonate buffer pH 8, and incubated for 1 hour at RT. Then, a 0.1 M phosphate buffer saline solution pH 7.0 containing 0.02% (v/v) Tween 20 (PBST) was pumped inside the system in order to remove the non-specifically adsorbed HRP molecules. The resulting biosensor systems were filled with PBS and stored at 4 °C until use.

A fourth biosensor approach was developed by direct adsorption of the HRP enzyme on the intact PDMS surface of the microsystem. It has been previously reported that proteins easily physisorbed on the surface of PDMS due to its high hydrophobicity.[13] In this work, the PDMS system was incubated in a 1 mg ml^{-1} HRP solution in 0.05 M carbonate buffer pH 8 for 1 hour at RT. Then, the system was rinsed in PBST and stored at 4 °C, in the same fashion as above.

Finally, solutions of 0.1 M acetate buffer pH 5.5 containing 0.5 mM ABTS and increasing concentrations of H_2O_2, from 0.1 μM to 100 μM were sequentially pumped inside each PhLoC. The resulting response was detected by using a LED working at a wavelength of 430 nm (spectrally very close to the previously mentioned first absorption band of $ABTS^+$), and measuring the spectral response using a microspectrometer (OceanOptics HR4000).

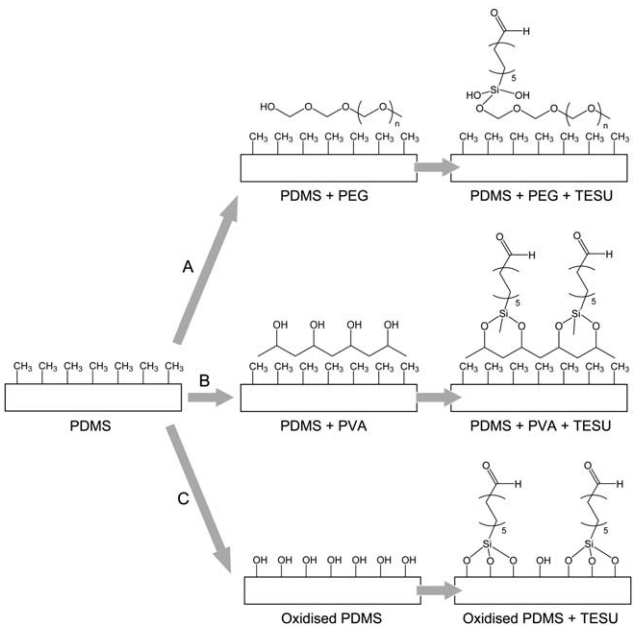

Fig. 2 Scheme of the different modification approaches tested for the biofunctionalisation of PDMS.

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Stability of the microsystems

The microsystems were stored in PBS at 4 °C for over two months. The stability of the immobilized enzyme was studied by repeating the calibration curve in a H_2O_2 concentration range from 0.1 μM to 1.53 μM. The slope (sensitivity) of the calibration curve was plotted over time for comparative purposes.

Results and discussion

Each one of the three different approaches for the modification of PDMS was chosen with the aim of providing the surface with a different density of hydroxyl groups. The PVA adsorption process could give rise to a higher proportion of these groups comparing with the PEG adsorption one, taking into account the chemical structures of both polymers. As for the chemical oxidation process of the PDMS surface, the conditions were set following an optimisation study, which showed that higher concentrations of H_2O_2 and HCl in the solution or longer incubation times gave rise to the PDMS surface degradation. As for the selection of the TESU aldehyde-terminated silane, this molecule enabled the straightforward covalent immobilisation of the biomolecule receptor without the need of using cross-linking molecules.

Structural characterisation of the modified PDMS surfaces

A rough but rapid estimation of the degree of modification of the PDMS surface following every step of each modification protocol was firstly provided by contact angle measurements. Given the hydrophobic nature of the intact PDMS surface, it was apparent that the introduction of chemical functional groups would give rise to a decrease of the contact angle value. The PVA-based approach was the one that appeared to induce the most significant changes on the PDMS surface. The contact angle of a native PDMS surface was found to be 114.57°, which is a similar value to that reported in previous studies.[27] Following the PVA modification step, it decreased to 102.47°. However, the contact angle did not change when PEG was adsorbed. This may be related to the higher density of hydroxyl groups that PVA contains in its linear structure compared with PEG, which only presents two hydroxyl groups at both ends of its chain. This facilitates the incorporation of a higher number of silane molecules during the silanisation step, which indeed gave rise to a further decrease of the contact angle on the PVA-modified surface to 96.9°, whereas no difference in this value was measured with the PEG-modified samples. The chemical oxidation approach induced a change in the contact angle value to 110.75°, which appeared to be not significant since such a value was not altered following the silanisation process. The light chemical oxidation of the PDMS surface that took place using the $HCl/H_2O_2/H_2O$ solution may account for such small differences in the measured contact angles. All these values were plotted in a bar graph, which can be found in the ESI‡ (Fig. S2‡).

XPS analysis was carried out to make a fine estimation of the density of hydroxyl and aldehyde groups introduced on the PDMS surface during the different modification steps. Table 1 shows the atom content in percentage values extracted from the XPS survey scan. An increase in the carbon content occurred following the PVA modification step, from 43.38% to 51.74%, while the Si percentage decreased from 36.90% to 27.05%. This is a clear indication of the adsorption of PVA molecules on to the PDMS surface. These changes were smaller in the samples prepared by the other treatments. High resolution spectra of the C1s region were also recorded. After the deconvolution of the C1 signal (Fig. S3–S5, ESI‡), new peaks could be detected that were related to each step of the PDMS modification processes. The native PDMS presents a unique peak that corresponds to the carbon atom of the methyl group, with a binding energy of 284.90 eV. After the first modification steps carried out to introduce –OH groups on the PDMS surface, a new peak was observed in all cases with energy of 286.50 eV, which is ascribed to C–O bonds (Table S1, ESI‡). Both PVA and PEG molecules contain this bond, but PEG has it in lower quantities, thus giving a smaller signal in the XPS spectra. In the case of the modification by chemical oxidation, Si–OH groups must have been introduced on the PDMS surface, instead of C–O bonds. However, it is reported that the oxidation of PDMS could give rise to –OH groups bound to the carbon atom of its methyl groups ($–CH_3$).[28] This may be responsible for the appearance of such a peak in the XPS analysis of the chemically oxidized samples. Nevertheless, its signal is significantly lower than that of the PVA-modified surface. Additionally, a new peak was observed in all samples further silanised with TESU. This peak is ascribed to the C═O bonds being part of the aldehyde end group of the silane molecules. Again, the highest density of this group appeared in the PVA-modified PDMS. From these results, it can be assumed that the PVA-based modification process seems to be more effective for the introduction of chemical functional groups on the surface of PDMS, which enabled the attachment of biomolecule receptors and thus the fabrication of biosensors.

The topographic and phase images obtained by AFM (Fig. 3) show that the unmodified PDMS surface is topographically flat as well as structurally and chemically homogeneous. In all cases, the modified surfaces upon each modification step exhibit from slight to dramatic variations in topography and composition, depending on the applied procedure. Branch-like structures can be observed on the PVA-modified surfaces (also shown in Fig. 3), which are likely to be related to the linear structure of the PVA polymer chains randomly adsorbed on to the PDMS. Furthermore, the PDMS surface significantly changed upon the silanisation process with TESU and a honeycomb-like polymeric structure was imaged (last two images of Fig. 3). Here, a polymerisation process could have taken place among the TESU molecules starting at those ones attached to the PDMS surface, which could somewhat act as nucleation points for the growing of such a polymer layer eventually covering the entire substrate. The adsorption of PEG did not seem to affect the topography of the PDMS substrates (Fig. S6, ESI‡). By contrast, the further silanisation process with TESU appeared to generate homogeneously dispersed dots, which are likely to be related to TESU-based polymer structures generated at specific positions where an isolated –OH group, coming from the adsorbed PEG molecules, appeared. As pointed out above, such big differences between PVA and PEG-modified surfaces could be explained taking into consideration that PEG contains a much lower density of –OH groups than PVA in their respective structures. These results are in good agreement with those obtained by XPS and contact angle

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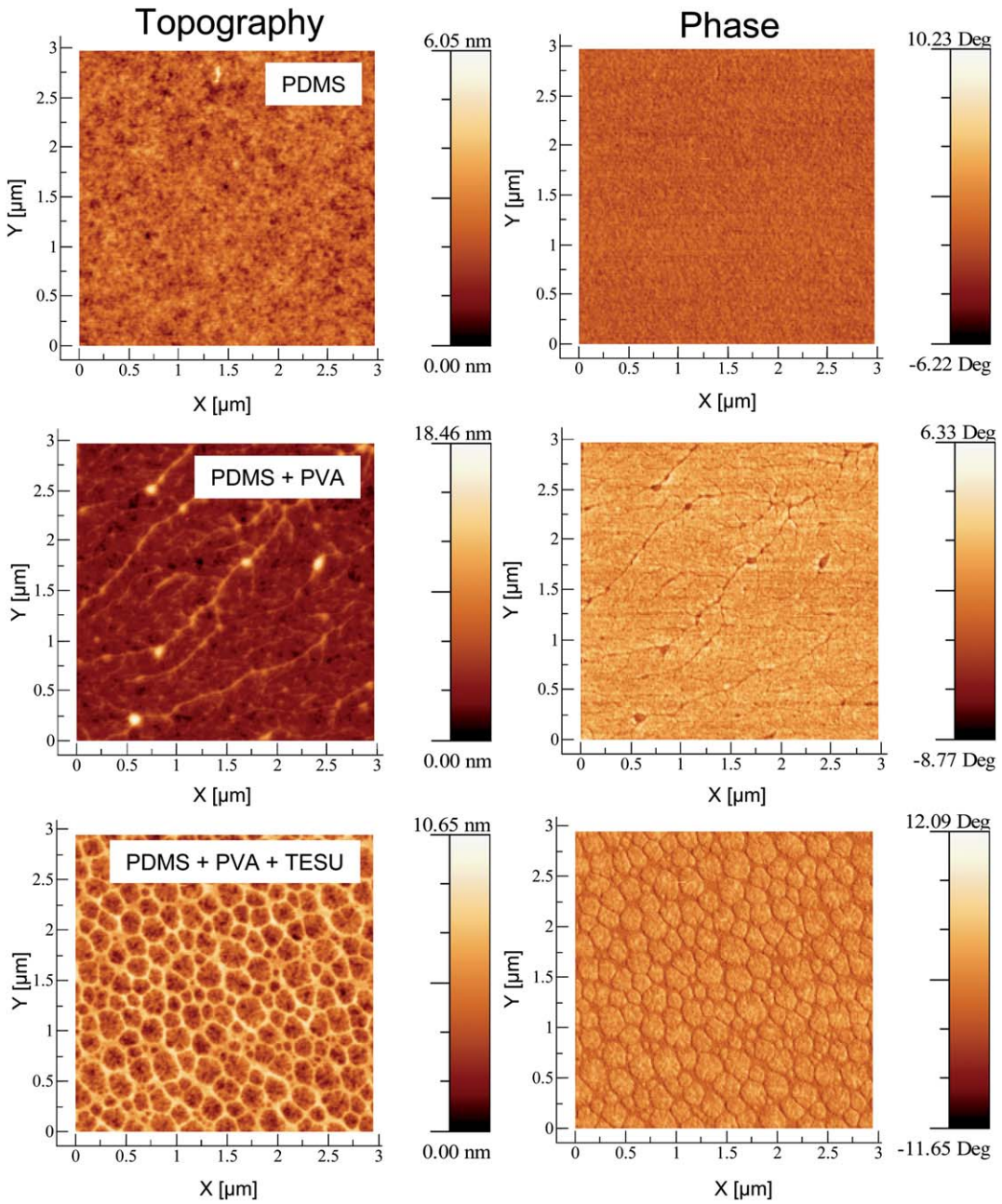

Fig. 3 Topographic and phase AFM pictures of the intact PDMS surface, after modification with PVA and after the silanization process.

measurements. Regarding the chemical oxidation process, AFM images show a surface roughness increase compared with that one of the native PDMS (Fig. S7, ESI‡). However, no significant differences were observed upon the silanisation process. These results indicate that the chemical oxidation process generated a very low density of –OH groups on the PDMS surface and so the further silanisation with TESU appeared to slightly change the topography of these samples.

Analytical performance of the different biosensor approaches

Once the HRP receptor was immobilised on the different modified-PDMS surfaces, the analytical performance of the resulting biosensor approaches was tested in H_2O_2 solutions in a concentration range from 0.10 µM to 100 µM. The corresponding calibration plots (Fig. S8, ESI‡) showed a linear increase in the measured absorbance at 420 nm up to a H_2O_2 concentration of 24.2 µM. Therefore, a linear fitting was carried out in this range and the analytical parameters were calculated (Table 2). No significant differences were observed among the different approaches. Nevertheless, the estimated error was higher for the adsorption approach, this being related to the lack of control of the adsorption process itself and the reported instability of the adsorbed molecules when carried out repeated measurements with the same PhLoC. A lowest limit of detection around 0.10 µM H_2O_2 was attained, this being between 10 and 100-fold lower than those values previously reported with similar analytical systems based on the use of a HRP receptor.[19,29]

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Table 1 Atomic percentages of the different surfaces extracted from the XPS survey scans

Sample	Atomic concentration (%)	
PDMS	O1s	19.71
	C1s	43.38
	Si2p	36.90
PVA	O1s	21.21
	C1s	51.74
	Si2p	27.05
PEG	O1s	19.05
	C1s	43.85
	Si2p	37.11
Oxidation	O1s	20.09
	C1s	44.92
	Si2p	34.99
PVA + TESU	O1s	21.29
	C1s	50.69
	Si2p	28.02
PEG + TESU	O1s	19.13
	C1s	45.36
	Si2p	35.51
Oxidation + TESU	O1s	20.26
	C1s	44.25
	Si2p	35.48

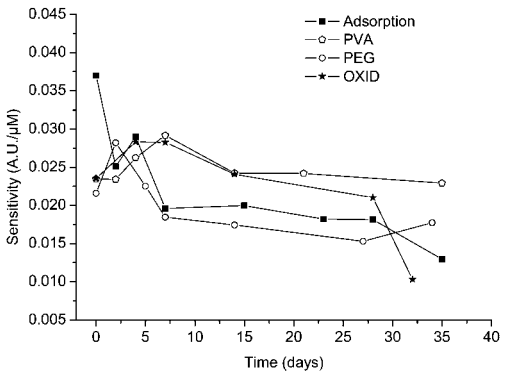

Fig. 4 Operational stability of the different PhLoC approaches measured as the sensitivity of the calibration plots sequentially recorded over a 35 day period.

Additionally, the sensitivity of the modified PhLoC improved 150 times when compared with another reported application using the same system.[22]

Finally, the operational stability of the PhLoC was systematically tested for over a month. Fig. 4 shows the shift in the sensitivity values with time. Two different trends were observed. Those devices based on the adsorption and PEG-modification processes show a rapid decrease in the sensitivity values during the first week of operation while those based on PVA-modification and chemical oxidation processes remained more stable for at least one month. However, a clear shift towards lower sensitivity values was observed for the oxidation approach after one month of operation while it was estimated that the PVA-based one retained 82% of its initial sensitivity after two months of operation.

Overall, from the results presented above, the PVA and oxidation approaches gave rise to the PhLoC systems with a better analytical performance in terms of reproducibility and stability. Nevertheless, the structural characterisation together with the analytical studies clearly indicate that the PVA-based approach is the one to be chosen considering the apparent higher density of chemical functional groups introduced on the PDMS surface and the longer stability of the resulting biosensor device.

Table 2 Analytical parameters of the four different biosensor approaches

Modification	Sensitivity[a]/a.u. μM^{-1}	LOD[ab]/μM	r
Adsorption	0.017 ± 0.003	0.12 ± 0.08	0.996
PEG + TESU	0.019 ± 0.001	0.28 ± 0.08	0.990
PVA + TESU	0.019 ± 0.001	0.14 ± 0.08	0.996
Oxid. + TESU	0.021 ± 0.005	0.10 ± 0.01	0.998

[a] Mean values and corresponding standard deviations of the parameters extracted from three calibration curves recorded with different devices in three consecutive days are represented. [b] LOD calculated following the 3σ IUPAC criteria using the lowest order of the linear concentration range from 0.1 μM to 1.53 μM.

Conclusions

Different chemical modification processes that enabled the introduction of hydroxyl groups on the surface of PDMS and the further attachment of a silane molecule for the selective immobilisation of a biomolecule receptor are reported. A thorough structural and analytical characterisation clearly indicated that the modification with PVA polymer is the most suitable approach for the biofunctionalisation of PDMS PhLoC systems. These processes do not require any specific instrumentation, thus enabling their easy and rapid implementation in chemical and biological laboratories working with PDMS microfluidic systems.

Acknowledgements

This work has received funding from the European Research Council under the European Community's Seventh Framework Programme (FP7/2007-2013) / ERC grant agreement no. 209243. The authors thank Dr Manuel Gutierrez for his kind help with the AFM study.

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Published on 18 February 2011 on http://pubs.rsc.org | doi:10.1039/C0AN00941E
Downloaded by University of York on 28 October 2012

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