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XPS and AFM Characterization of the Enzyme Glucose Oxidase Immobilized on SiO_2 Surfaces

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A process to immobilize the enzyme glucose oxidase on SiO_2 surfaces for the realization of integrated microbiosensors was developed. The sample characterization was performed by monitoring, step by step, oxide activation, silanization, linker molecule (glutaraldehyde) deposition, and enzyme immobilization by means of XPS, AFM, and contact angle measurements. The control of the environment during the procedure, to prevent silane polymerization, and the use of oxide activation to obtain a uniform enzyme layer are issues of crucial importance. The correct protocol application gives a uniform layer of the linker molecule and the maximum sample surface coverage. This result is fundamental for maximizing the enzyme bonding sites on the sample surface and achieving the maximum surface coverage. Thin SiO_2 layers thermally grown on a Si substrate were used. The XPS Si 2p signal of the substrate was monitored during immobilization. Such a signal is not completely shielded by the thin oxide layer and it is fully suppressed after the completion of the whole protocol. A power spectral density analysis on the AFM measurements showed the crucial role of both the oxide activation and the intermediate steps (silanization and linker molecule deposition) to obtain uniform immobilized enzyme coverage. Finally, enzymatic activity measurements confirmed the suitability of the optimized protocol.

Introduction

Over the past few years there has been a growing interest in creating microbiosensors, integrated within microelectronic circuits. Biosensors are considered an everyday tool to monitor both our environment[1] and our health.[2] The increasing interest in biosensors by the community led the research to find innovative devices that could satisfy both the needs of mass production and accurate analysis. For these reasons, many research groups have used Si-compatible technology as an innovative platform to create biosensors.[3,4] Many goals will be achieved using Si-based materials: (i) a low-cost and mature technology, fundamental for mass production; (ii) device miniaturization, implying reduced molecular diffusion path, faster kinetics, and an improvement of the analytical performance of the device;[4,5] (iii) the possibility to create microstructured devices achieving complex functions, e.g., micro-total-analysis systems;[6] (iv) the integration of the electronics and/or photoelectronics needed for detection on the same chip.[7] Moreover, miniaturization opens the field of in vivo physiological monitoring; it implies lower reagent consumption, hence minimized sample volumes, lower energy consumption, and less space requirement (sensor portability).[8]

For the above-mentioned reasons, the studies related to Si-based biosensors are considered an interesting research field.[3] The goal of this study was the covalent immobilization of a biological molecule on an SiO_2 surface followed by its full characterization by techniques already employed in microelectronic research. The biosensor creation processes must be characterized and optimized using techniques that will not cause the denaturation of the biological molecule or, even more important, its complete destruction. We used the enzyme glucose oxidase (GOx),[9,10] extracted from *Aspergillus niger*. It is 160 kDa homodimeric globular protein, with a tightly bound ($K_a = 1 \times 10^{-10}$) flavin adenine dinucleotide (FAD) per monomer.[9] The overall dimer dimensions are $6.0 \times 5.2 \times 7.7$ nm^3 measured by X-ray crystallography.[10] The enzyme catalyzes the oxidation of β-D-glucose to D-glucono-1,5-lactone and hydrogen peroxide, using molecular oxygen as the electron acceptor.[11] This enzyme is usually employed when the glucose concentration in the blood must be measured; hence, Gox-based micro-biosensors[12] would have immediate applications in monitoring diabetes.[13]

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Biological molecules can be anchored on SiO_2 surfaces using different methods,[4,12,14−20] depending on the biological molecule to immobilize. The most used approach is the formation of covalent bonds between the molecule and the solid surface, often using linker molecules.[4,12,14−16,18,19] The immobilization procedure must be optimized to obtain the maximum surface coverage and to prevent the denaturation of the biological molecule and/or the loss of its specific properties, e.g., for an enzyme its enzymatic activity.

X-ray photoelectron microscopy (XPS)[21] and atomic force microscopy (AFM)[22] can be considered complementary techniques to monitor surface modifications and to fully characterize the immobilization protocol of biological molecules on SiO_2 surfaces. XPS is a powerful technique to provide chemical bonding information and molecular composition of the materials lying on the sample surface or in the first layers beneath it.[21,23,24] XPS measurements have recently been used to characterize glutamate dehydrogenase immobilization on the surface of silicon dioxide[25] and to optimize the immobilization procedure. They have also been used to determine the amount of glucose oxidase entrapped in polymeric films.[26] However, AFM is the best technique to monitor the morphological surface structure.[22] It is widely used to characterize both biological molecules in solutions[10] or those immobilized on solid surfaces, mainly mica or gold.[14] Moreover, it has been used to study nanoscale adhesion, friction, and wear properties of streptavidin biomolecules on silica.[27] In this work, we used XPS and AFM techniques to monitor the surface modification during GOx immobilization on SiO_2 to obtain the maximum surface coverage.

Experimental Section

Surface Preparation and Measurement Techniques. Silicon dioxide layers were thermally grown on 6 in. Si wafers through an oxidation process at 950 °C for 30 min in O_2 atmosphere. The oxidation time was chosen to grow a thin oxide layer, 8 ± 1 nm thick (based on uncorrected ellipsometry measurements). The SiO_2 surface roughness was 0.15 nm, in agreement with literature data for thermal oxide.

XPS analyses were carried out using a Kratos AXIS-HS spectrometer. The Mg $K\alpha_{1,2}$ of 1253.6 eV was used at the conditions of 10 mA and 15 keV with a pass energy of 40 eV. During the analysis the residual pressure in the chamber was 10^{-7} Pa.

AFM measurements were performed in air at room temperature using an XE 150 by PSIA scanning probe microscope operated in the noncontact mode. Different samples subjected to different steps of the immobilization protocol were characterized. For each sample, AFM scans (typically 1×1 μm) were carried out on several surface positions to check the surface uniformity. The two-dimensional (2D) isotropic power spectral density (PSD) and the surface root-mean-square (rms) roughness were calculated for all the AFM maps representative of the different protocol steps. The surface rms roughness is defined as the standard deviation of the heights (*Z* values) expressed in nanometers in a *XYZ* three-dimensional AFM map. The PSD is mathematically defined as the Fourier transform of the autocorrelation function between surface points. It is a plot of spectral power as a function of spatial wavelength or frequency. Spectral power is expressed in units of length squared and divided by a two-dimensional spatial frequency (reciprocal of length squared); hence, PSD is expressed in units of length to the fourth power. PSD is isotropic; it is an average taken over all directions in the AFM map. rms roughness is related to PSD since rms is the square root of the integral of PSD over wavelength or frequency interval. Since PSD transforms the surface's real spatial features into a histogram of spatial frequencies, a PSD plot is especially useful for investigating a trend in surface features. In fact, surfaces consisting of regular features yield PSD plots comprised of relatively few frequencies. Even for irregular surfaces, interesting information can be obtained from the PSD spectrum. Surfaces with small and sharp features yield a broad PSD plot with long tails extending to higher spatial frequencies. On the other hand, large and rounded features on the surfaces produce high-frequency tails having a relative weight much lower than the one of surfaces with small and sharp features. Hence, the characteristic surface features of the differently prepared samples could be compared by comparing the 2D isotropic power spectral densities.

Immobilization Protocol. The enzyme immobilization procedure consists of four steps: (1) oxide activation; (2) silanization; (3) linker molecule deposition; (4) enzyme coupling. In the first step the samples were immersed in an ammonia and hydrogen peroxide water solution (SSC). The second step consisted of a treatment in vapors of 3-aminopropyltriethoxysilane (APTES). The APTES chemical formula is $NH_2-(CH_2)_3-Si(OC_2H_5)_3$. After silanization, the samples were cured under vacuum at 80 °C for 40 min. The linker molecule deposition was carried out using glutaraldehyde (GA), 2.5%, in phosphate buffer solution (PBS). GA is a linear molecule ($CHO-(CH_2)_3-CHO$) with one aldehydic group (CHO) at each end. Samples were immersed in PBS solution containing GOx, 2 mg/mL, overnight at room temperature (RT).[15,28] All samples were dried under a gentle nitrogen flow and stored at RT until measured, which was always performed within 2 days from preparation. As reference, some samples were simultaneously treated with water. Other references were prepared by processing some samples with only one immobilization step. Hence, samples that underwent only one step are indicated as "only-APTES", "only-GA", and "only-GOx", while samples that underwent the various protocol steps in water are labeled as "reference". Finally, the protocol steps were evaluated measuring the samples after the first step, the second step (up-to-APTES), the third step (up-to-GA), or after the full process (labeled as "full").

Enzymatic Activity. GOx activity was determined by measuring the amount of H_2O_2 formed using a spectrophotometric commercial glucose assay kit, purchased from Megazyme. In the presence of peroxidase (POD), hydrogen peroxide, produced by GOx during glucose oxidation, participates in a reaction involving *para*-hydroxybenzoic acid and 4-aminoantipyrine (both provided in the kit) and a quinoneimine dye complex is formed, which is measured at 510 nm.[29]

Materials. APTES, GA solution (grade II), glucose oxidase (GOx, type X-S, *Aspergillus niger*), disodium hydrogen orthophosphate (Na_2HPO_4), sodium dihydrogen orthophosphate (NaH_2PO_4), and hydrogen peroxide at 31.3% (H_2O_2) were purchased from Sigma Chemical Co., St. Louis, MO. Cloridric acid (HCl), fluoridric acid

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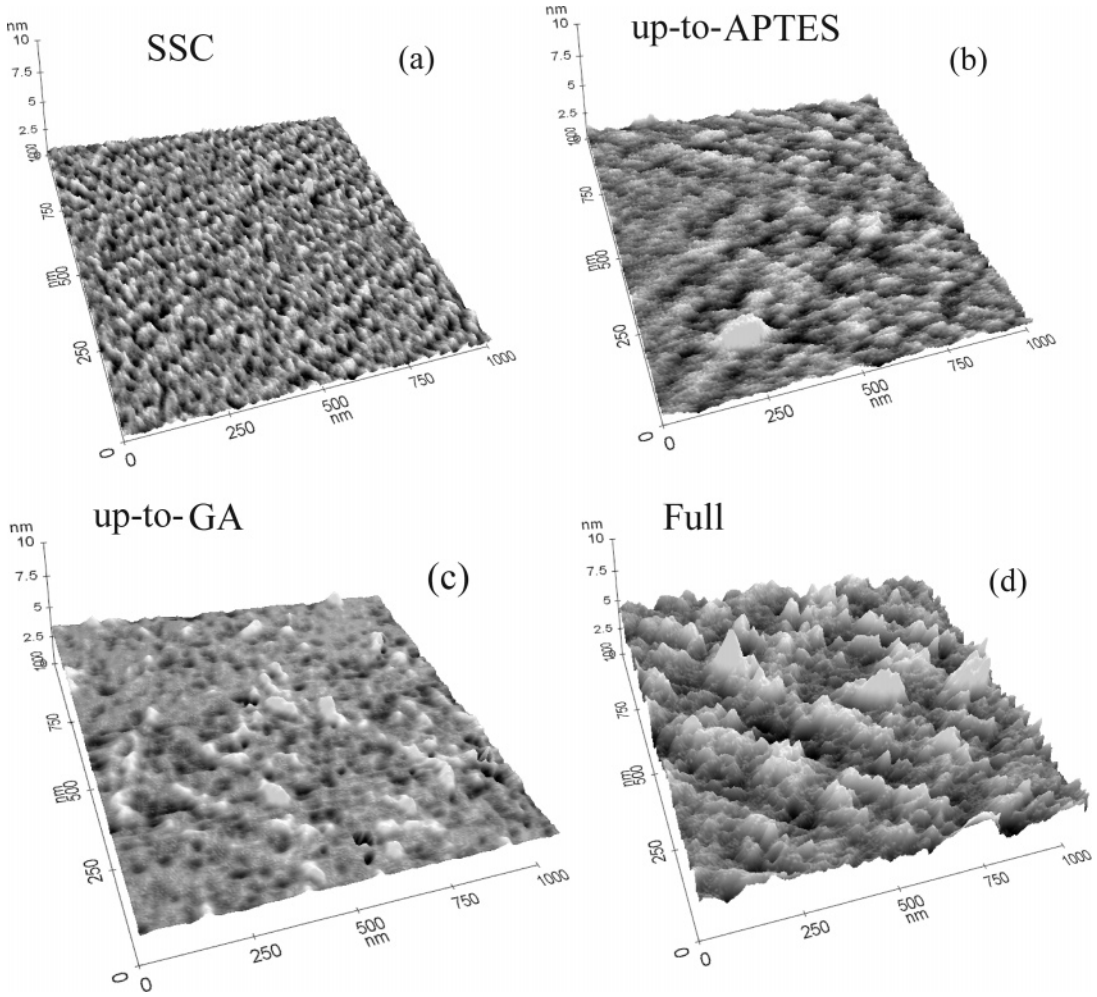

Figure 1. AFM images of (a) SSC, (b) up-to-APTES, (c) up-to-GA, and (d) full samples. The vertical scale is 10 nm while the horizontal scales are both 1 μm. Higher peaks appear lighter on a gray scale.

40% (HF), ammonia at 25−28% (NH_3), acetone (CH_3H_6O), and isopropyl alcohol ($CH_3CH(OH)CH_3$) were purchased by Carlo Erba Reagenti, Milan, Italy. The water used was deionized, MilliQ, having 18 MΩ resistivity.

Results and Discussion

Samples having SiO_2 thin layers, see Experimental Section, were processed according to the immobilization procedure previously reported.[15,28] The first step, oxide activation with SSC solution, was used to clean the surface from adventitious contaminations and to increase the number of "active" sites for silanization. On these samples, both XPS and AFM were carried out and, as expected, no detectable modifications were observed on the surface after this step, both measurements being the same as the reference sample. In particular, the XPS survey spectrum of the reference sample, treated with water alone (data not shown), exhibited Si (Si 2s and Si 2p) and O signals and a very small C signal, due to adventitious contamination. The rms roughness obtained from AFM measurements on the same sample (data not shown) was ∼0.15 nm, typical of thermally grown silicon dioxide.

The AFM analyses for all the intermediate steps of the immobilization process are reported in Figure 1. Figure 1a shows the result on the sample that underwent only oxide activation (labeled as SSC). An rms of 0.14 nm was obtained, indicating that the surface did not undergo macroscopic modifications during the first immobilization step, as previously mentioned. The average peak to valley height was 0.3 nm, while the "pore" radius was, on average, 0.03 nm. These values were the same, within the experimental errors, to the values observed for a thermal Si oxide. The fraction of the total area covered by pores was 22.1%.

The second step, silanization, allowed us to functionalize the sample surface with amino groups that easily bonded the linker molecule, glutaraldehyde, if silane polymerization did not occur. AFM measurements of this sample, shown in Figure 1b, indicate a surface rms value of 0.22 nm. We used the same vertical scale in all three-dimensional images to allow a direct comparison of the samples. It should be noted that the APTES layer, if the surface is properly functionalized, has a physical thickness of 0.8 ± 0.1 nm.[30] The AFM results suggested a good sample coverage. The highest peaks observed in Figure 1 are due to APTES polymerization. Samples that did not undergo oxide activation showed more polymerized sites (as shown in Figure 8b), suggesting that a lower number of active sites was available for GA immobilization, and a much higher rms value.

Other interesting results, obtained with our experimental protocol were provided by the contact angle measurements, reported in Table 1. Reference samples, both unprocessed and processed in water, had a contact angle of ∼56°, typical of SiO_2, when adventitious contaminations were present on the oxide surface.[31,32] Samples that underwent the APTES process, without

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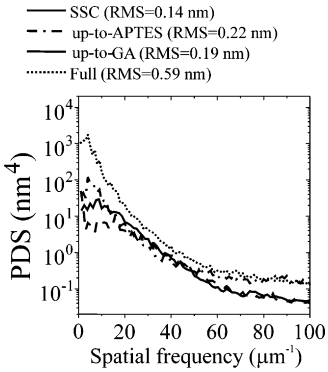

Figure 2. Comparison of the PSD spectra of the reference, up-to-APTES, up-to-GA, and full samples. The respective rms values are also reported.

Table 1. Contact Angle Measurements on SiO_2 Surfaces; First Column Indicates Sample ID

sample	contact angle	sample	contact angle
reference	56°	up-to-APTES	81°
up-to-SSC	50°	only-APTES	77°

oxide activation, had a contact angle of ~77°, typical of silanized surfaces (75−80°).[31] This result is indicated in the literature (see ref 31) as obtained from samples having a uniform silanization on the surface. Our results demonstrate that greater hydrophobic behavior can be obtained if the samples undergo oxide activation before silanization (up-to-APTES). In this case, the oxide activation had a double effect: it reduced the surface contaminations and allowed the formation of more surface sites available for silanization (OH terminations). As a macroscopic result, the surface coverage with silane groups was higher and the hydrophobic character was more pronounced (contact angles of 81°).

During immobilization, one glutaraldehyde (GA) end bonds the amino group of the silane, while the other end can bond the protein amino group. When the GA was deposited on the sample surface, only small modifications of the surface morphology occurred (Figure 1c). In fact, the surface rms, as measured by AFM, was 0.19 nm, barely distinguishable from the data extracted from the previous sample measurements. The data clearly indicated that the surface did not undergo relevant modifications, detectable by AFM. Finally, AFM measurements carried out on a sample that underwent the full immobilization process are shown in Figure 1d. A clear modification of the surface occurred due to enzyme immobilization, with the appearance of peaks having the same height and good distribution on the sample surface. The rms value for this sample was 0.59 nm. The qualitative observations of the surface evolution following the four steps of the immobilization protocol are quantitatively expressed in Figure 2, where the PSD spectra are compared on a log-linear scale. The most evident difference between the PSD spectra associated with the first three steps and the spectrum associated with the last one is in the low spatial frequency region. In particular, at a spatial frequency of ~5 μm^{-1}, the PSD value for the fully processed sample was about 20 times higher than the one of the sample processed up-to-APTES. These high spectral power components at low frequency correspond to the high and broad peaks in the AFM image of the fully processed sample (Figure 1d).

A complete explanation of the observed morphological modifications, and their absence after the first three immobilization steps, was provided by XPS analysis. As an example, the XPS Si 2p spectral regions for some immobilization steps are summarized in Figure 3. Also XPS data showed differences

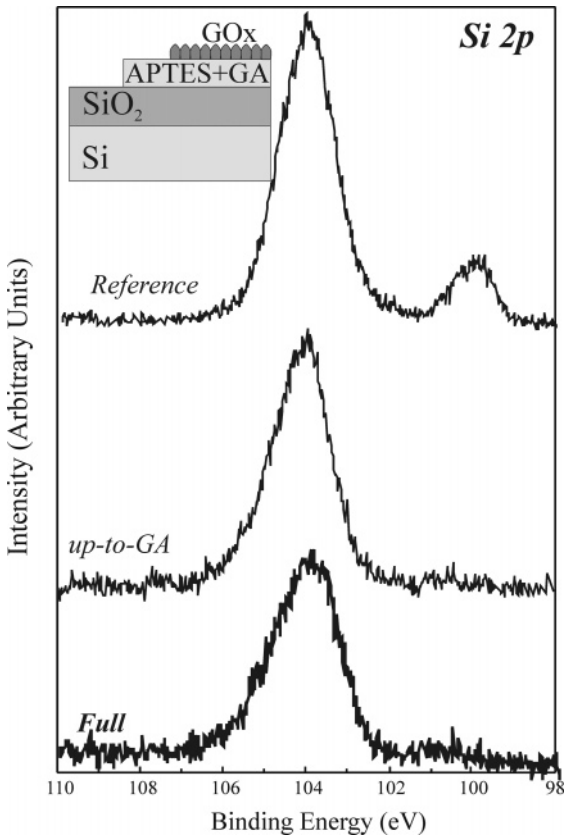

Figure 3. XPS Si 2p spectral regions of the reference (top), up-to-GA (center), and fully processed (bottom) samples. The insert shows the progressive surface coverage during the immobilization steps.

between the fully processed samples and those stopped before the final step. The inspection of the C 1s peak (see ref 28) allowed us to have conclusive proof of the enzyme deposition on the sample surface, while the study of the Si 2p spectra provided information on the surface coverage. The reference sample (treated with water alone, Figure 3, top spectrum) showed two components with binding energies of 99.7 and 104 eV, assigned to Si^0 and SiO_2, respectively.[23,24] The SiO_2 component was centered at 104 eV instead of 103.4 eV, as expected for silicon oxide, due to differential charging between the SiO_2 layer and the Si substrate.[33] The SiO_2 layer thickness ($d = 6.5$ nm) was calculated from the intensities of the Si 2p peak components assigned to the Si^0 and SiO_2 following the procedure reported in ref 28.

The progressive depositions of APTES, GA, and GOx produced extra layers on the SiO_2 surface whose thickness increased with the process step. The final film deposited on the Si substrate, given by both the SiO_2 and the organic layers (APTES + GA + GOx), had a thickness that completely shielded the Si 2p substrate signal, as can be seen from the spectrum of the fully processed sample of Figure 3 (bottom). The observation of the up-to-GA sample (center) clearly shows that such a signal was already fully shielded after GA deposition (SiO_2 + APTES + GA, see insert), as demonstrated by the presence of the SiO_2 layer component and the absence of any signal in the region where the Si^0 should have been. Two conditions must be fulfilled to have a complete substrate shielding: (i) a film thickness above the photoelectron escape depth and (ii) the uniformity of the layer. According to literature data,[30] since APTES and GA are small molecules, uniform thin layers on the SiO_2 surface can be

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Table 2. Relative Abundance (% of Atoms) of the Various Elements (C, O, N, and Si) for the Measured Samples

sample	C	O	N	Si
reference	7.7	63		29.3
up-to-APTES	28.8	41	0.6	28.5
up-to-GA	51.2	26.5		22.3
full	50.8	28.8	5.5	14.8
only-GA	30.7	42.4		26.8
only-GOx	39.4	35.2	6.9	18.5

obtained if their immobilization protocol is optimized. In particular, the physical thickness of an APTES monolayer is 0.8 $\pm$ 0.1 nm, while the total thickness of the APTES + GA layer is 1.5 $\pm$ 0.1 nm. As a result, when the third step of the immobilization protocol was carried out, the film on the Si substrate had a total thickness of 8.0 nm (6.5 nm of SiO_2 + 1.5 nm of organic compounds). The progressive layer deposition is summarized in the insert of Figure 3. The Si substrate signal intensity is attenuated by two layers (SiO_2 + organic), based on the calculations reported in ref 28. Our results confirmed that the immobilization protocol was correctly optimized to provide a uniform GA layer ready for GOx deposition, as demonstrated by XPS data. In fact, the up-to-GA sample did not exhibit the Si 2p substrate signal to confirm the uniform GA film deposition. Since APTES tends to polymerize (see later), a uniform GA distribution is not easily obtained. The final issue to be addressed is layer uniformity. In fact, it could be said that a thick, but not uniform, layer could produce the same results.

The atomic surface concentrations of the most interesting samples are compared in Table 2. The reference sample showed a small carbon concentration due to adventitious contaminations (7.7%). With the use of immobilization protocol, the carbon content on the sample surface increased, approaching about 50% when organic molecules (both GA and GOx) were deposited on the surface. In the reference samples treated only with GA and only with GOx, the carbon percentage strongly decreased compared to that of the respective processed samples, going from 51% of the functionalized samples (up-to-GA and full) to 30% and 39% for the only-GA and only-GOx samples, respectively. These results suggest that when the sample surface is not opportunely functionalized, the surface coverage by GA and GOx is not uniform. In fact, the reduction of carbon content in those samples was accompanied by an increase of Si and O, the SiO_2 elements. Due to the sensitivity limits of the XPS technique, phosphorus and the other functions of the FAD enzyme groups were not detected.

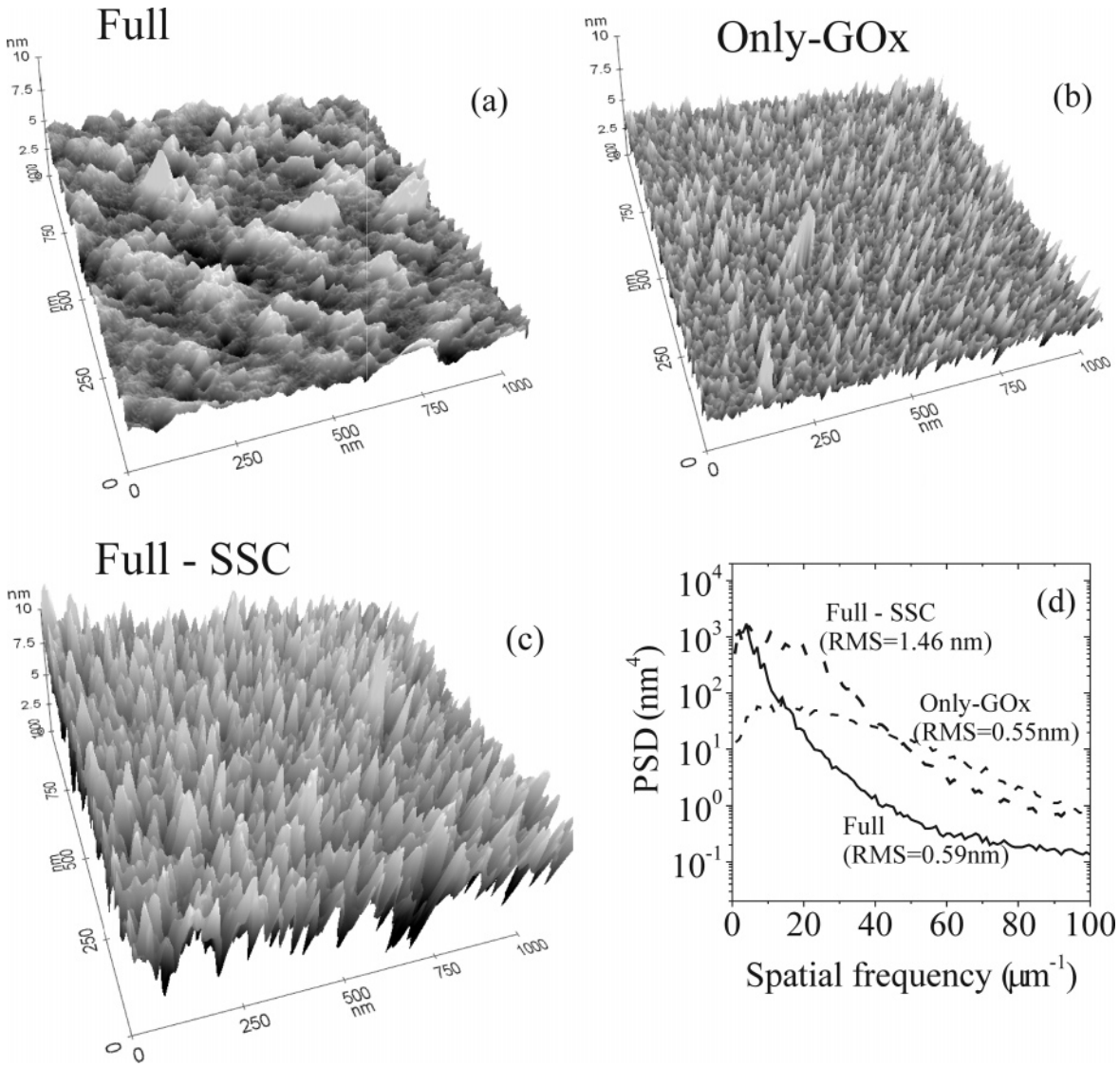

Figure 4. AFM image of the (a) full, (b) only-Gox, and (c) full without SSC samples. The vertical scale is 10 nm while the horizontal scales are both 1 μm. Higher peaks appear lighter on a gray scale. (d) Comparison of the PSD spectra of the three samples. The respective rms values are also reported.

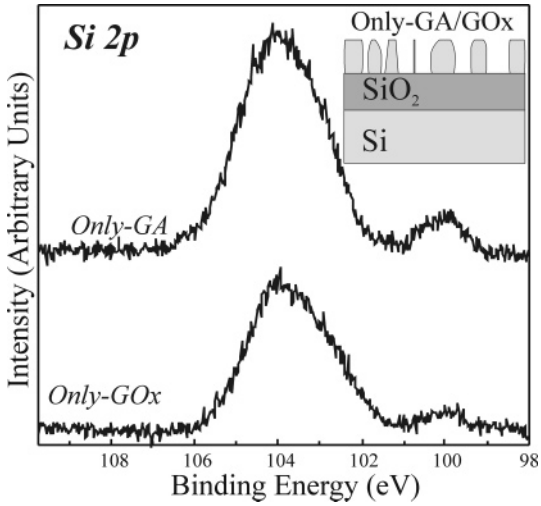

Figure 5. XPS Si 2p spectral regions of the only-GA (top) and only-GOx (bottom) samples. The insert shows the nonuniform organic film coverage.

To fully confirm the previous conclusions, the other reference samples were measured. As an example, the comparison between the surface morphologies and the PSD spectra for the fully processed sample (full, Figure 4a) and the sample not subjected to the second and third immobilization step, i.e., only-GOx (Figure 4b), is shown in Figure 4. The only-GOx sample is an interesting marker to observe the GOx dimension as detected by AFM. Most of the enzyme was, of course, washed away, as expected, but some molecules formed nonspecific bonds, due to Van der Waals forces, with the sample surface and resisted the washing procedure. The two samples (full and only-GOx) exhibited similar rms roughness values (0.59 and 0.55 nm, respectively). However, in spite of this, the surface morphologies appeared dramatically different. High and broad features were present in the fully processed sample, while smaller and sharp features were observed in the only-GOx sample. It is quite interesting to note that the peak heights in this sample were around 5 nm, due to the enzyme dimension. This apparent contradiction is explained by comparing the PSD spectra in Figure 4d. The only-GOx sample exhibited smaller PSD values at the lowest spatial frequencies ($<20\,\mu m^{-1}$), while a broad and high tail was present at the highest frequencies. This tail is, obviously, associated with the distribution of small and sharp features in this sample.

The surface coverage was evaluated through the XPS Si 2p signals of the only-GA (top) and the only-GOx (bottom) samples reported in Figure 5. The insert shows a schematic representation of the sample cross section. The nonuniform film is drawn on the SiO_2 surface. When only-GA was deposited on a nonfunctionalized sample, a nonuniform layer was formed (see insert), due to the lack of amino groups to bond to the surface. As a result, the substrate Si signal was not shielded in the regions where the GA did not bond and a final appreciable signal was observed.

This result was confirmed by the atom percentage of Si and O in this sample shown in Table 2 (see before): they are higher than those in the up-to-GA sample. The XPS spectrum of the only-GOx sample provided the final conclusion of this work. Even if GOx is a thick protein, as shown by AFM measurement, when it is deposited without the proper surface functionalization, a nonuniform layer is formed. As a result, the substrate signal is reduced, but not suppressed, from a nonuniform GOx layer. To obtain a rough estimation of the covered surface fraction, a simple calculation was carried out, using the formulas reported in ref 28. The ratio between the intensity of the two Si peaks

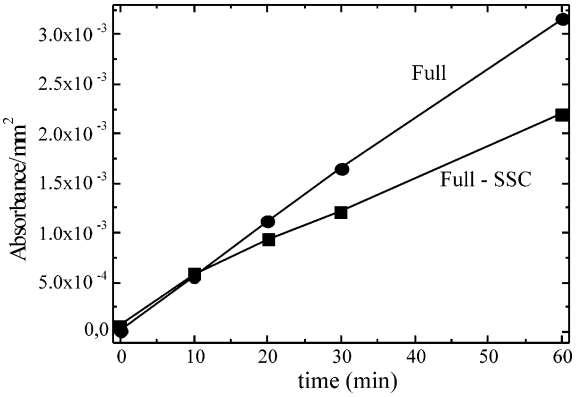

Figure 6. Glucose oxidase activity as a function of time for a sample fully processed without SSC (squares) and with SSC (circles).

(I_{Si}/I_{ox}) was calculated as a function of the covered surface fraction, assuming that, when present, the organic layer (d_{org}) had a constant thickness (see insert in Figure 5). From this hypothesis, a covered fraction of 80% ($I_{Si}/I_{ox} = 0.069$ and $d_{org} = 5.5$ nm) was determined for the only-GOx sample, while a covered fraction of about 50% ($I_{Si}/I_{ox} = 0.997$ and $d_{org} = 1.5$ nm) was determined for the only-GA sample. The reference sample exhibited a $I_{Si}/I_{ox} = 0.171$, in agreement with the calculated value of 0.164.

Some considerations must be made on the immobilization protocol, particularly on the importance of the first two immobilization steps: oxide activation and APTES functionalization. As mentioned before, oxide activation is fundamental for achieving the best surface coverage. If the sample did not undergo this step, the surface coverage was not uniform. It can be easily seen from the comparison of Figures 4a and 4c, where the AFM images of fully immobilized GOx samples with and without oxide activation, respectively, are compared. The rms roughness of the sample not subjected to oxide activation (1.46 nm) was much higher than the rms roughness of the fully processed sample (0.59 nm), as can be deduced from the broad PSD spectrum. This indicated that surface coverage, also in this case, was not uniform and the peak heights were not all the same, indicating that there were regions where, probably, the GOx did not bind to the surface. The estimation of the covered surface fraction provided a value of 90% ($I_{Si}/I_{ox} = 0.091$ and $d_{org} = 7$ nm). Nevertheless, also in this case the enzyme presence can be detected through the glucose reaction and H_2O_2 production, as shown in Figure 6. In fact, the activity of the immobilized enzyme was monitored using a spectrophotometric assay. GOx activity was monitored on both the sample that underwent the four steps of the full immobilization protocol (with SSC) and the sample that underwent the protocol without SSC (labeled full-SSC). The enzyme was active after immobilization and, as expected, an increase in the enzymatic activity was observed when the oxide activation was carried out before silanization. By comparing the absorbance of the SiO_2-immobilized enzyme with the free enzyme in solution, we estimated the concentration of active GOx on the SiO_2 samples to about 0.002 U mL^{-1}. More extensive data on enzymatic activity are reported in refs 29 and 34.

It should also be pointed out that a correct APTES surface functionalization plays a crucial role in the final surface coverage. If the APTES polymerization occurs, e.g., in the presence of water vapor, a completely different surface morphology is obtained. XPS analysis did not show any difference between the spectra of a sample correctly silanized (up-to-APTES) and of

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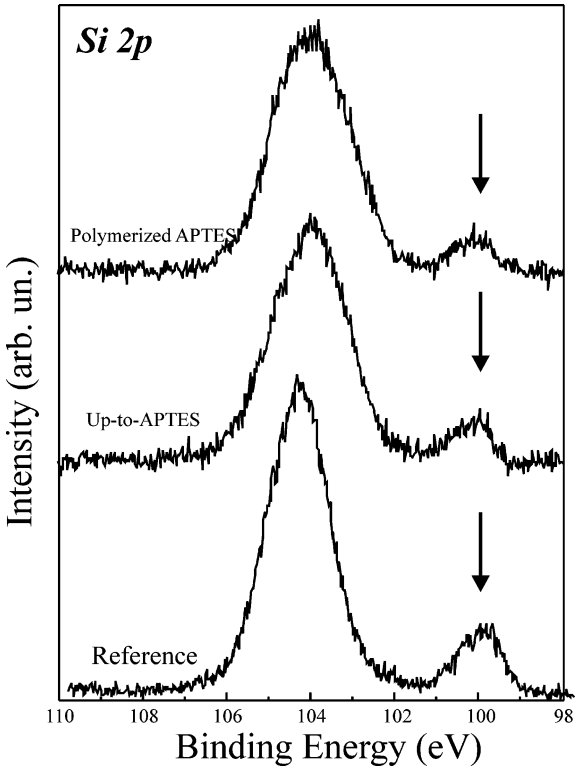

Figure 7. XPS Si 2p spectral regions of the polymerized APTES (top), up-to-APTES (center), and reference (bottom) samples.

one polymerized since the chemical shifts in the Si 2p were not distinguishable in our experiments. The XPS Si 2p spectra at the APTES step for a sample correctly functionalized (center) and one where the APTES has polymerized (top) were compared to the reference sample (bottom) in Figure 7. The spectrum of the surface with polymerized APTES was not very different from that of a correctly functionalized one. The major indication of the incorrect silanization process was obtained by AFM measurements. The comparison between the AFM image of a correctly silanized surface (Figure 8a) and a polymerized APTES sample (Figure 8b) clearly explain our comment. The AFM image in the polymerized APTES sample exhibited broad and high peaks, causing an rms roughness (0.66 nm) 3 times higher than the one of the correctly treated sample (0.22 nm). Moreover, in the polymerized sample, PSD values in the low spectral frequency region were 2 orders of magnitude higher than those in the correctly treated one (see Figure 8c). These peaks were due to the APTES polymerization of the groups. They tend to bond to each other, forming large agglomerates on the sample surface. These last results show the importance of the first two immobilization steps.

The optimized protocol was used for the creation of simple, MOS-like, biosensor structures.[35]

Summary

This paper reports a successful immobilization procedure of the glucose oxidase enzyme on an SiO_2 surface. The samples were characterized using XPS, contact angle, and AFM measurements. The use of these techniques, often providing complementary information, allowed us to fully explore both the efficacy and efficiency of the immobilization protocol used. We successfully immobilized the enzyme on the SiO_2 surface. In particular, a significative increase in the AFM rms was obtained after full immobilization. PSD data extrapolated from AFM measurements allowed us to acquire quantitative data on the biological layer uniformity.

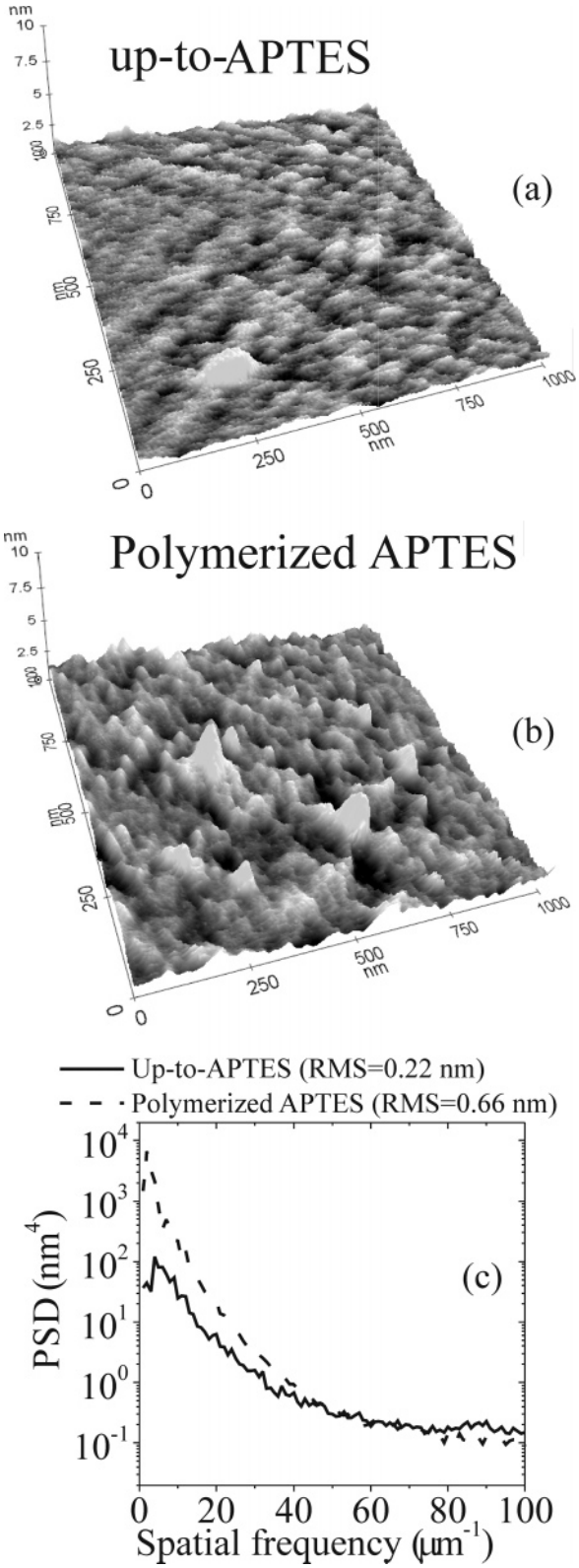

Figure 8. AFM of the correctly processed up-to-APTES sample (a) and of a sample where the APTES was polymerized. The vertical scale is 10 nm while the horizontal scales are both 1 μm. Higher peaks appear lighter on a gray scale. (c) Comparison of the PSD spectra of the two samples. The respective rms values are also reported.

(35) Libertino, S.; Fichera, M.; Aiello, V.; Lombardo, S.; Scandurra, A.; Renis, M.; Sinatra, F. Accepted for publication in *Sens. Lett*.

AFM images and XPS Si signals, as well as surface compositions of samples representative of each step of immobilization protocol, show that the deposition of a uniform GA layer is crucial for GOx immobilization. This is the basic condition to obtain the maximum surface coverage with the enzyme deposition. AFM measurements allowed us to demonstrate the importance of oxide activation and of a correct silanization, while from a simple numerical calculation of the ratio I_{Si}/I_{ox} derived from the Si 2p XPS observations, a covered fraction of 100% was determined for the fully processed sample, 90% for the fully processed sample without SSC, and 80% for the only-GOx sample.

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