ACS APPLIED MATERIALS & INTERFACES

Surface Functionalization of Fluorine-Doped Tin Oxide Samples through Electrochemical Grafting

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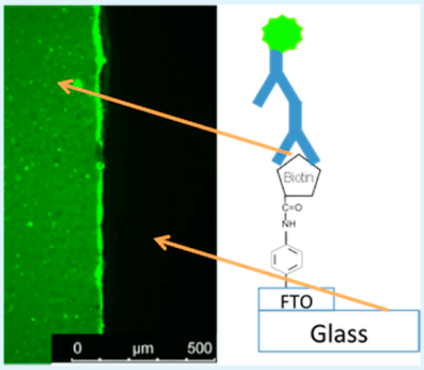
Supporting Information

ABSTRACT: Transparent conductive oxides are emerging materials in several fields, such as photovoltaics, photoelectrochemistry, and optical biosensing. Their high chemical inertia, which ensured long-term stability on one side, makes challenging the surface modification of transparent conductive oxides; long-term robust modification, high yields, and selective surface modifications are essential prerequisite for any further developments. In this work, we aim at inducing chemical functionality on fluorine-doped tin oxide surfaces (one of the most inexpensive transparent conductive oxide) by means of electrochemical grafting of aryl diazonium cations. The grafted layers are fully characterized by photoemission spectroscopy, cyclic voltammetry, and atomic force microscopy showing linear correlation between surface coverage and degree of modification. The electrochemical barrier effect of modified surfaces was studied at different pH to characterize the chemical nature of the coating. We showed immuno recognition of biotin complex built onto grafted fluorine-doped tin oxides, which opens the perspective of integrating FTO samples with biological-based devices.



KEYWORDS: *FTO, transparent conductive oxides, electrochemical grafting, biosensor, immunofluorescence assay, AFM phase imaging, wet etching*

INTRODUCTION

Transparent conductive oxides (TCOs) are materials that have been extensively used in the last years in various fields in which both transparency and high conductivity are required, like optoelectronic technology, for example, solar energy conversion,[1,2] electrochromic mirrors,[3,4] dye-sensitized solar cells (DSSCs),[5,6] display applications,[7−9] and advanced biological based technologies (for instance, optically transparent biosensors[10−13]).

The ongoing research is driven by the attempt to achieve long-term stability in modifying TCO properties to optimize performances.[14,15] It is well known in fact, that the possibility of broadening the energy band gap of these semiconductors could in principle increase electron mobility[15] enhancing overall efficiency. Also, TCO modification is linked to maximize energy conversion,[16] to increase light trapping efficiency (haze),[17,18] to improve adhesion of coatings in devices,[19,20] to limit the unwanted light absorption portion of solar spectrum,[21,22] and to facilitate biointegration in an environmentally and biocompatible friendly supply.[23] There are two main experimental strategies for TCO modifications: (i) bulk modification and (ii) surface modification.[24]

In the first case, bulk modification is limited to extrinsic doping of semiconductors to alter intrinsic wide band gap of materials:[25,26] the deterrent of using this approach is related to the high costs required for homogeneous and high quality bulk modification since not conventional techniques has to be used (i.e. magnetron sputtering or state solid electrochemistry) for integrating small atoms or ions into the crystallographic structure.

On the other hand, it has been demonstrated that properties modification can be obtained by simply tuning interfacial properties by selective deposition of smart molecules (for example, SWCNTs,[27] TiO_2,[28] or graphene sheets[29]): the main drawback of surface modification is the limited chemical reactions that can take place on almost all TCO surfaces. Such reactions involve silanes,[31−33] phosphonic acids,[34] or other small molecules.[24,30] There are evidences, for instance, that indium tin oxide (ITO) modified surface increases electron transfer properties,[35] optimizes light trapping,[36,37] allows tuning surface roughness and tribological properties via any plasma

Received: August 9, 2013
Accepted: October 30, 2013
Published: October 30, 2013

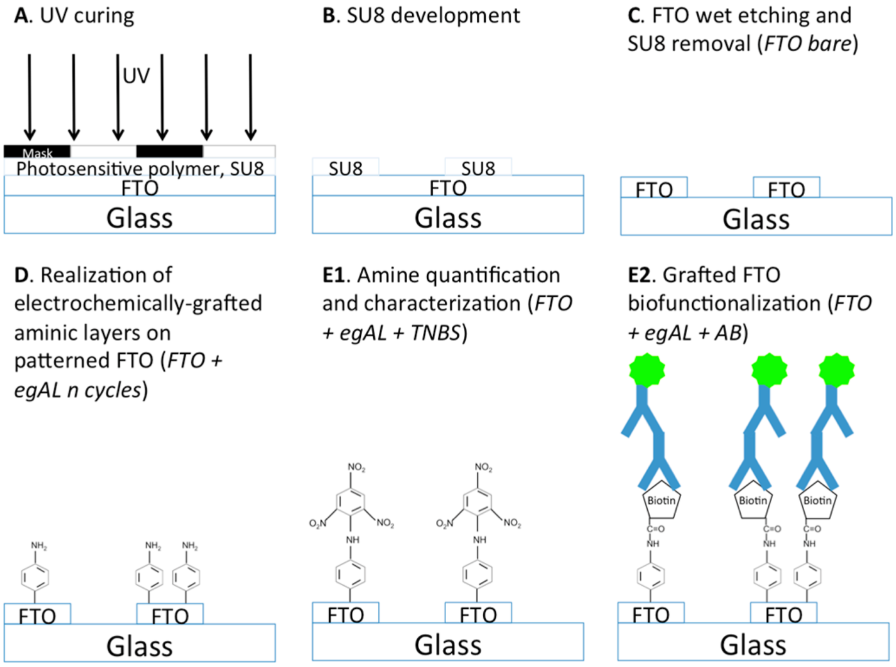


Figure 1. Schematic representation of the modification occurred at FTO surfaces in this manuscript. Panels A−C show FTO patterning procedure and in particular: A refers to UV curing of the photosensitive polymer used for creating FTO patterned mask; B shows its development; and C shows its removal and FTO selective wet etching procedure (see Experimental Section for details). D shows the electrochemically grafted layer production, whereas E1 shows the amine quantification step via TNBS covalent immobilization, and E2 shows the immunofluorescence assay realization for validating the biofunctionalization.

activation.[38,39] However, there are still unsolved problems mainly related to long-term and thermal stability, reproducibility of the treatment, chemical surface homogeneity after modification, and controlled protocols for monolayer formation.[30]

Although ITO is the most used TCO, its cost is continuously rising because of the low availability of indium,[40] prompting the researchers to find new materials with comparable advanced optoelectronic properties.[41] In addition, ITO is pH sensitive, and it rapidly dissolves in diluted acidic media.[42,43] This behavior is useful in creating transparent micropatterned circuits but strongly limiting in applications where *click-chemical* reagents cannot be used.

Among the other new fascinating optomaterials, fluorine-doped tin oxide (FTO) is an emerging TCO that overcomes all of these limitations: the electronconductivity is achieved by fluorine atoms (instead of oxygen vacancies) that give stability to the lattice to chemical attack, also at low pH, resulting in a nontoxic, fully biocompatible, and cost-affordable TCO material.[44−46] Because of its novelty, the early applications are still limited to thin film photovoltaics,[47] dye sensitized solar cells (DSSCs),[48] and photoelectrochemical based devices.[49] Recent studies on self-cleaning surfaces made by FTO are growing in number.[50] In literature, only a few recent papers concerning the covalent modification of FTO surfaces are available.[49,51,52] However, the limitation to FTO surface modification is that occurring chemical reactions are not selective. So far to our knowledge, no works concerning selective and efficient surface modification are reported.

For this reason, in this work we aim at investigating the chemical functionality to FTO electrodes in order to selectively and covalently immobilize a controlled amount of molecules with long-term stability and reproducibility. The selectivity of the modification is achieved by use of electrochemical grafting technique. Besides the other techniques, electrochemical grafting is a well-established experimental strategy for modifying surfaces that allows to combine selectivity and high yields. By this approach, a surface chemical reaction is triggered by an opportune polarization of the electrode immersed in a precursor solution: in this way, carbon electrodes,[53] gold and other noble metals,[54,55] and lastly ITO surfaces[56−58] have been successfully modified. It is noteworthy to underline that Gooding et al.[57] have recently shown how grafted layers on ITO surfaces are more durable and electrochemically stable in a wide range of potential with respect to chemically adsorbed monolayers. On the other hand, in our knowledge, no works concerning electrochemical grafted FTO samples are available as it could match electrochemical selectivity with TCO smart optoelectronical properties.

Softlithographic techniques are used for patterning FTO surface, whereas cyclic voltammetry (CV), X-ray photoelectron spectroscopy (XPS), and atomic force microscopy (AFM) are used for characterizing quantitatively the grafted layers. Since the electrochemical barrier effect of modified surfaces is also studied at different pH, an attempt to comprehend the chemical nature of the coating is presented.

Figure 1 reports the overall strategy. After FTO preparation and patterning (Figure 1A−C), the electrode is electrochemically grafted on the surface by controlling degree of modification (Figure 1D). Then, the amount of grafted molecules is characterized by cyclic voltammetry by means of amine-specific redox species bound to the surface (Figure 1E1) and, finally, immuno-recognition of biotin is used for biointegration validation (Figure 1E2).

■ EXPERIMENTAL SECTION

FTO Patterning and FTO Cleaning. All chemicals and reagents are from Sigma-Aldrich, Italy, if not differently stated. Prior to

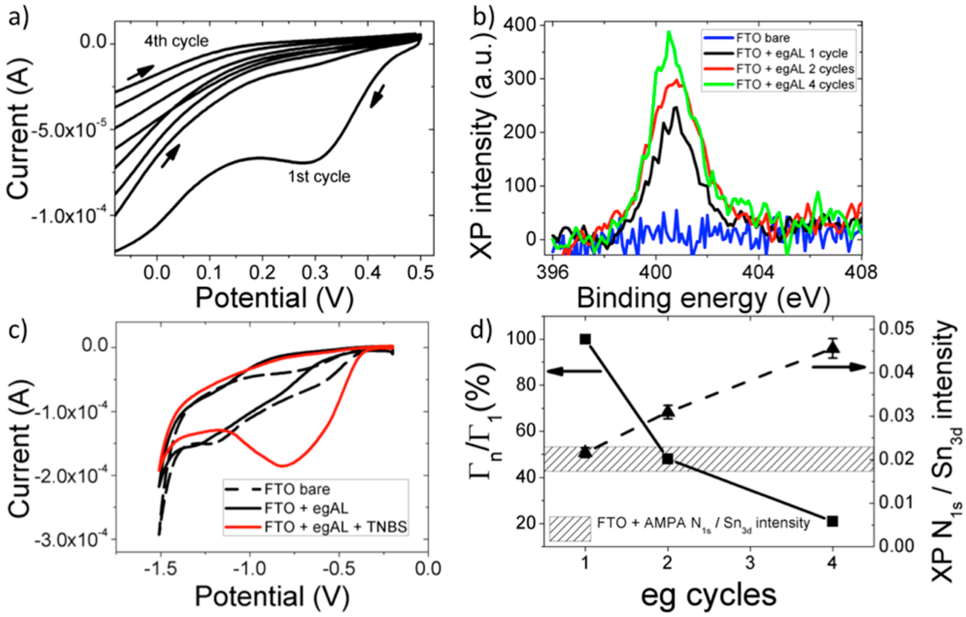


Figure 2. Electrochemical and XPS characterization of modified FTOs. (a) CV showing FTO surface modification via electrochemical grafting of in situ diazonium cations. Arrows represent scan direction. (b) N_{1s} photoemission spectra of modified FTOs at different electrochemical grafting cycles. (c) CV showing irreversible electrochemical reduction of nitro groups anchored to FTO + egAL samples. (d) Comparison between electrochemical surface coverage (defined as footnote) of nitro groups (left *y*-axis) and XP N 1s/Sn 3d intensity ratio (right *y*-axis) after different electrochemical grafting cycles. Shaped area represents XP N 1s/Sn 3d intensity for FTO + AMPA electrode.

modification, FTO-coated sodalime glass (7-10 Ohm/□) is subjected to a strong cleanness protocol constituted by several treatments under sonication (isopropanol, methanol and acetone, 15 min each). Then samples are dried with nitrogen flux and stored until used under nitrogen. The FTO patterning is achieved by creating a 5 μm acidic resistant layer using SU8-2005 (Microchem Corp., USA). This layer is realized starting from a polycarbonate mask designed with AUTOCAD software. The photoresist is deposited on the surface of clean FTO and UV-cured following procedure described by supplier.[59] FTO is covered with a thin film of Zn powder in order to start FTO acidic erosion.[60] So, FTO can be selectively wet etched by dipping on the masked surface into a strong acid mixture (HCl 37% 5M + $FeCl_3$ 0.5 M) at room temperature until all FTO is removed, realizing different geometries on the surface of millimeter size. Successively, the sample is rinsed with Milli-Q water until neutral pH is reached. Then the photoresist is removed by a solution of commercial organic remover (Remover PG, MicroChem Corp., U.S.A.). A final rinsing with ethanol and Milli-Q water follows. FTO clean bare electrodes are then prepared by UV-ozone (UV/Ozone ProCleaner, BioForce Nanosciences Inc., U.S.A.) plasma treatment for 20′, followed by acetone and Milli-Q water rinsing and nitrogen dry.

Electrochemical Measurements. An electrochemical cell is realized using FTO patterned bare electrode as working electrode (1 cm-disk), a Pt counter electrode (Mettler Toledo, Spain) and a Ag/AgCl sat reference electrode (Amel Instruments, Italy). Kapton tape (2M, Italy) is used for insulating electric contacts. Supporting electrolytes (buffers) used are reported as follows. Supporting electrolyte for TNBS (2,4,6-trinitrobenzenesulfonic acid) detection is PBS 1× (Life Technologies, Italy). Redox probe used is 0.5 mM hexaamineruthenium(III) (RU6A). Scan rate is 50 mV/s.

The pH was controlled using the following buffer: pH 2, 100 mL 0.2 M KCl + 26.0 mL 0.2 M HCl; pH 3, 98.2 mL 0.1 M acetic acid and 1.8 mL 0.1 M acetate sodium; pH 4, 84.7 mL 0.1 M acetic acid +15.3 mL 0.1 M acetate sodium; pH 5, 35.7 mL 0.1 M acetic acid +64.3 mL 0.1 M acetate sodium; pH 6, 5.2 mL 0.1 M acetic acid +94.8 mL 0.1 M acetate sodium; pH 7, 100 mL 0.1 M KH_2PO_4 + 58.2 mL 0.1 M NaOH.

FTO Modification. The grafting solution (aryldiazonium cation solution) was prepared as follows: 5 mM $NaNO_2$ and 1 mM *p*-phenylendiamine (final concentrations) were dissolved in a 0.5 M HCl aqueous solution.[61] The latter is stirred for 5′ at dark in order to guarantee the formation of products, then transferred into the electrochemical cell. A cyclic voltammetry was performed for creating grafted aminic layers on top of FTO surface. Three different grafted samples were realized by scanning the potential in the negative direction at constant rate (0.1 V/s) between 0.5 V and -0.9 V: 1 cycle, 2 cycles and 4 cycles modified FTOs were obtained. The samples were vigorously washed with Milli-Q water to eliminate unwanted absorbed molecules and dried with nitrogen gas. TNBS was covalently attached to immobilized amine by simply immersing modified FTOs in 1 mM TNBS solution for 1 h at pH 9.6 (100 mL 0.025 borax added to 22.2 mL 0.1 M NaOH). Then samples were copiously rinsed with Milli-Q water and dried with nitrogen.

FTO samples modified with aminomethylphosphonic acid (AMPA) are treated as follows. Bare FTO electrodes were immersed in 1 mM AMPA for 24 h to achieve modification of the surface and treated under nitrogen flow for 24 h at 120 °C.[57] Then samples were rinsed with ethanol and Milli-Q water, then dried with nitrogen gas.

Modified samples were stored in nitrogen box for several months and weekly tested to characterize long-term stability of modification. After 4 months, FTO-modified electrodes shown restored voltammetric behavior.

XPS Characterization. The XPS data reported in the present study were obtained at room temperature in normal emission configuration using a non monochromatized Mg Kα radiation (1253.6 eV) and a VG Escalab MkII electron analyzer. Before recording photoemission spectra, the samples were degassed overnight in UHV (ultra high vacuum, pressure lower than 10^{-8} mbar). In order to discriminate between the real chemical grafting and the physical adsorption of the nitrogen species on the FTO substrate, prior to XPS measurements, the samples were annealed at 300 °C using a pyrolytic boron nitride heater and the temperature was monitored using a K type thermocouple directly clipped onto the sample surface.

AFM Characterization. A morphological characterization of the sample surfaces was carried out by AFM (NT-MDT, Russia) in non-contact mode, scanning sample regions of area ranging from 3×3 to 20×20 μm^2. Phase images were acquired together with topology recordings to investigate the distribution of material surface characteristics. AFM tips were NSC11 Ultrasharp silicon cantilever (Mikromasch, Bulgaria).

Immunofluorescence Assay. Thirty seven milligrams of biotin are added to 30 mL of water reaching a final concentration of 5 mM; 25 mM *N,N'*-diisopropylcarbodiimide (DPC) and 50 mM *N*-hydroxysuccinimide (NHS) were added to the solution to activate carboxylic functions of biotins. The reaction was left to complete for 1 h at 50° C under sonication. Finally, the solution was cooled at ambient conditions and the grafted FTO samples were immersed for 24 h at room conditions in the prepared biotin solution. A rinsing step with acetone and Milli-Q water for removing unbound molecules followed. Samples are then dried with nitrogen gas.

A primary anti-biotin antibody produced in goat was used for the detection of biotin on surfaces. All other materials for immunofluorescence labeling were purchased from Life Technologies, Italy. FTO surface was washed in phosphate buffer (DPBS) and passivated with a 3% w/v solution of bovine serum albumin (BSA) for 30 minutes. Surface was then incubated for 1 h at room temperature with anti-biotin antibodies diluted in passivation solution at 40 mg/mL. FTO was extensively washed with DPBS and incubated again for 45 minutes with a fluorescein-labelled anti-goat antibody. FTO was extensively washed again with DPBS and fluorescence emission at 512 nm was detected by a DMI6000B Leica microscope with an excitation filter at 450-490 nm.

RESULTS AND DISCUSSION

Prior to modification, FTO surfaces are treated with UV-ozone plasma cleaner to achieve a hydrophilic surface as shown in previous works concerning other TCOs:[38] this procedure is very important because hydrophilicity is related to efficiency in electrochemical grafting procedure. In fact, by comparing wet base piranha treatment to UV-ozone plasma cleaning, an increased amount of OH groups is detected for UV-ozone plasma treated samples (Figure S3 of Supporting Information). When the grafting solution is placed into the electrochemical cell, the Open Circuit Potential (OCP) value is increased of about 0.6 V demonstrating a high amount of charged molecules ($-N_2^+$ species) in the Nernst layer. Three different grafted samples are realized by cyclic scanning the potential in the negative direction between 0.5 V and -0.9 V: 1 cycle, 2 cycles and 4 cycles modified FTOs are obtained (the samples are labelled as FTO + egAL *x* cycles, where *egAL* means "electrochemically grafted aminic layers" and *x* represents the number of CVs used for modifying the electrodes). As shown in Figure 2a, during the 1st scan a well-defined irreversible reduction peak is visible at 0.3 V representing the aryldiazonium cations reduction at the electrode. Samples treated with 2 and 4 cycles exhibit very small and not well-defined reduction peaks .

This experimental evidence suggests that covalent grafting takes place from the first scanning cycle and it is consistent with similar works reported in literature for different working electrodes.[56,61,62]

First of all, to characterize grafted electrodes in terms of quality of chemical species, we performed XPS analysis. Grafted FTO substrates show a N 1s peak centered at 400.6 eV (Figure 2b), at the same binding energy position observed for the aminomethylphosphonic acid functionalized surface, used for comparing grafted layers with chemically adsorbed ones (Figure S1 of Supporting Information). The binding energy values are in agreement with literature values for amine group.[63] After the first cycle, there is a significant increase in the N 1s/Sn 3d photoemission peak ratio, which saturates at the value expected for a fully covering monolayer (~0.02 referred to FTO + AMPA samples). During the following cycles the grafting of N species is much more limited.

This seems to indicate that during the first cycle, a highly efficient grafting of the diazonium salts directly on the FTO surface takes place, while during the following cycles, we are dealing with a slower multilayer growth. However, the microscopic mechanism explaining the growth of multilayer films (either involving $-N{=}N-$ bonds, or aromatic ethers, or $-C-C-$ bonds)[43] is still debated and better resolved photoemission measurements would be needed in order to provide a final assessment, since the chemical shift between $-N{=}N-$ and NH_2 is very small.[63] Also, Electrochemical Impedance Spectroscopy analysis allows to highlight a different electrochemical behavior between AMPA functionalized samples and grafted ones. This difference attests that adsorbed layers have a less complex structure with respect to grafted ones, suggesting a multilayering growth mechanism in grafted samples (Figure S1 of Supporting Information).

To investigate whether the aminic layers upon grafting are available for chemical functionalization, a specific redox species is bound to the modified electrodes. It is noteworthy, in fact, that TNBS is quite sensitive to primary amines[55] and irreversible reduction of nitro groups can be monitored through CV at −0.7 V. Integrating the voltammetric curve, the indirect quantification of aminic functions can be obtained by varying surface FTO modification degree.[64] [The electrochemical surface coverage of nitro groups (i.e., the aminic surface coverage) used in the manuscript is here defined as the ratio between electrochemical surface coverage at the *n*th electrochemical grafting cycle (Γ_n) and the electrochemical surface coverage at 1st cycle (Γ_n): Γ_n/Γ_1.] The CVs in Figure 2c show the electrochemical detection of TNBS anchored to aminic layers upon grafting. An irreversible peak at about −0.7 V was found during negative scanning of modified grafted FTO samples. In contrast, although XPS and EIS have shown effective covalent modification of AMPA on FTOs, no covalent immobilization of TNBS was detected on these substrates. This result clearly confirms previous research concerning low stability and chemical accessibility of adsorbed layers on TCOs.[57]

The conclusion is that only grafted FTO samples show efficient surface chemical functionalization.

By comparing XPS and voltammetric surface coverage data as a function of electrochemical grafting cycles (Figure 2d), it is possible to highlight that increasing electrochemical grafting cycles we observe a decreasing of anchored TNBS (error <1%) and an increasing of XPS photoemission intensity. These outcomes confirm that the surface is already fully functionalized after the 1st cycle.

This outcome has strong impact on FTO applications, because for the first time, we demonstrated that a monolayer modification is necessary for achieving high yields of surface functionality: when electrode modification occurs by a multilayer growth mechanism, a diminution up to 80% of chemical functionality occurs. A possible explanation can be given by assuming that thick grafted films becomes self limiting to electron flow to the underlying FTO electrodes. In addition, the progressive cyclic dependent decreasing of the N groups exposed by the surfaces can be correlated to the growth of the successive layers .

To characterize the morphology of surface along grafting cycles, we perform AFM analysis. FTO UV-ozone treated samples are quite rough (about 35 nm of average roughness, see Table S1 of Supporting Information), and since the morphology is quite identical to that of modified FTOs (Figure

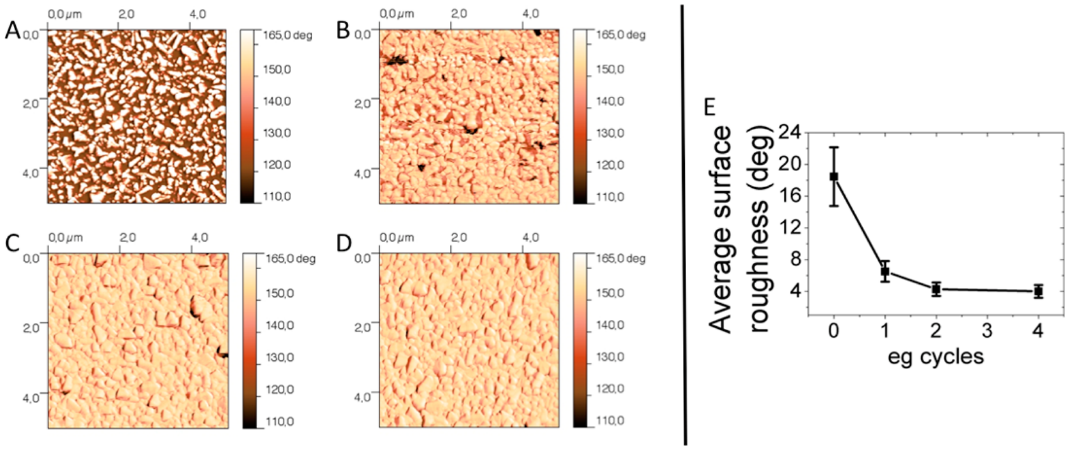


Figure 3. Phase imaging AFM results. A−D show phase images at different degree of modification (A, bare FTO; B−D, FTO + egAL cycle 1−4), whereas graph in panel E shows AFM phase median of degree distribution versus electrochemical grafting cycles. The first point refers to bare FTO sample. As shown, increasing electrochemical grafting cycles, a smaller average surface roughness of FTO samples is found.

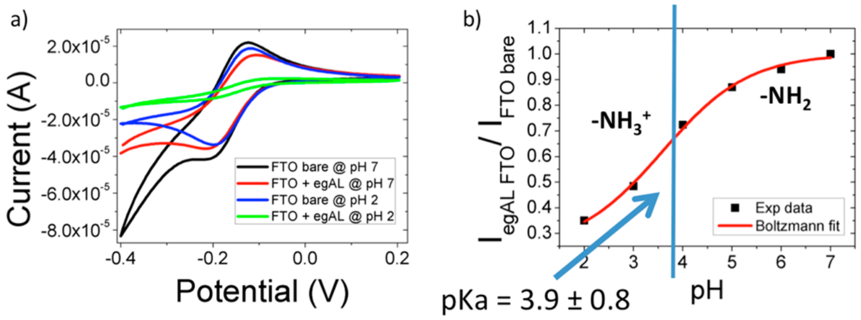


Figure 4. pKa determination of grafted chemical species on FTO surface. a) CV showing bare FTO and grafted FTOs at different pH solution in presence of a charged redox probe. Decreasing pH values, the electrochemical barrier effect becomes more relevant due to electrostatic repulsion between positively charged surfaces and redox probe. Redox probe: RU6A, 0.5 mM. See Experimental Section for buffers used. (b) Normalized current as function of solution pH: the pKa can be estimated as the flex of the fitted curve.

S2 of Supporting Information), our choice was to use non-contact phase imaging for studying the topography. In fig. 3A-D AFM phase images of samples at different degree of modification are shown. Bare FTO samples show relative low amount of features on the surface, related to native surface protrusions of the oxide due to UV-ozone treatment (Fig. 3A). After 1 cycle of modification, the number of features on the surface becomes bigger until a complete saturation of the surface takes place (Figure 3B). Further modification thickens the grafted film (Figure 3C−D) so that AFM tip cannot enter between the grafted layers and the oxide surface cannot be visible anymore.

This behavior is consistent with the electrochemical outcome suggesting the saturation of the surface after one cycle of grafting. Also, this outcome is quantitatively evidenced by roughness analysis of AFM phase images. Figure 3E shows average surface roughness versus electrochemical grafting cycles: increasing modification degree of the surface (i.e., number of grafting cycles), a diminution of roughness is achieved. This point is a key aspect in comprehending chemical functionality of multilayered samples: thus, concluding, a thicker film (Figure 2D) has low chemical functionality (Figure 2C) because of low surface roughness (Figure 3E) and, probably, because of self limiting behavior.

Simultaneous characterization both via XPS and AFM leads to better phenomena comprehension. This experimental strategy allows obtaining grafted films with accurate and reduced thickness. This aspect is relevant in treating modified optically transparent electrodes, in which bulk modification of the surface can be a significant drawback.

Now the problem shifts towards the production of versatile chemical active layers on the surface in order to realize FTO-based applications, justifying our next investigation concerned to the chemical interface properties. In particular, pKa knowledge of the top primary amine emerging from the surface is a fundamental value to know for performing further covalent assembly on FTOs. Also, its value can provide an a posteriori justification of growth mechanism. It is known that using charged redox probes, an electrostatic interaction between modified surface and redox centers is constituted at specific solution pH and this method has just been used for characterizing grafted layers on glassy carbon electrodes.[61] In the cited work researchers used the electrochemical barrier effect of the positive charged redox probe for investigating the effective electrode passivation because at low pHs the aminic functions are positively charged. Therefore, our contribution to the state of the art is to study pKa of FTO + egAL samples by tuning solution pH during CV in presence of a positively charged redox probe (hexaaminoruthenium(III), RU6A). On the contrary, if a non-charged redox compound is used (ferrocene methanol, FcMetOH), no significant barrier effects are evident as other reported because of non-covalent interactions between lattice oxygen and methanol (not shown).[58] In Figure 4a typical electrochemical outcomes are summarized for grafted samples at different pH: no substantial barrier effect is found at pH 7, whereas no faradic current is obtained at pH 2 for grafted samples, that is, the surface is become not accessible for RU6A. In Fig. 4b normalized faradic current defined as ratio between faradic current of grafted samples and faradic current at bare FTO as function of solution pH is reported: interestingly, experimental data can be fitted with a Boltzmann curve and a flex is found at 3.9 ± 0.8 that is consistent to an aniline-like adsorbed monolayer (soluble aniline pKa is about 4.5[62]). This result is quite the same for all degrees of modification, confirming the idea that a small film is realized on the surface of the electrodes.

As overall conclusion, grafted FTO samples display tunable chemical and electrochemical interfacial properties. First of all, pKa determination is of fundamental importance since it is related to the chemical accessibility to the surface for covalent and long-term modification. Its value is consistent with a monolayer of aniline directly bound to the surface. This result is in contrast with other findings in literature in which bigger values were found.[65] A possible explanation can be given by considering that in those works a bulk modification of the surface occurs (i.e., multilayering) because of a potentiostatic treatment. In such circumstances, chemical functionality of interface molecules can be strongly affected by thickness and also electroconductivity can be reduced due to the formation of a thick insulating layer on the top of the electrode. As a perspective, in our lab there are attempts to realize irreversible bonds by changing target molecules in order to tune chemical reactivity, without loosing electro-optical efficiency, which is strongly required in TCO-based applications.

Secondly, electrochemical response of FTO modified samples, is affected by what kind of redox probe species is used (i.e., by the non covalent interactions between molecules and grafted surfaces): our results show that a positively charging surface can be produced. For this reason, this work opens the possibility for the development of new opto-materials able to tune interfacial charge storage by simply modifying electron mobility towards the electrodes.

It is noteworthy to underline that transparency is a fundamental property of substrate materials for biological studies which are mostly based on optical imaging (such as bright phase contrast, fluorescence, or luminescence) of living systems. Typically, they require spatial proximity between cells and sensitive units (i.e., biosensors or immunoassays) to perform intracellular and continuative measurements. For this reason, FTO electrodes can be very useful in this perspective, because they combine high optical transparency and electroconductivity that can in principle allow to selectively modify glass substrates. So, once the chemical functionality of the modified FTO has been fully characterized, an immunoassay is realized to the proof of concept of selective biofunctionalization. Biotin, a vitamin interested in cell growth, is usually used in biochemical assays for protein conjugation as it is very reactive with avidin (K_d about 10^{-14} mol/L), so a great amount of biotinylated compounds are commercially available. In particular, ELISA tests require an extensive modification of the surface of the glass in order to obtaining an effective fluorescence yield. This work has shown how it is important to accurately control surface modification of TCOs in order to achieve good quality optical images. In Figure 5, an immunofluorescence assay is presented. Biotin is covalently coupled to FTO + egAL samples, whereas anti-biotin recognizes specifically biotin substrate; then, the secondary antibody that is modified with a fluorophore is specifically linked to the primary antibody. It is noteworthy to underline that no aspecific absorption of biotin/primary antibody is found on the surface of glass, demonstrating without any doubts high selectivity of the electrochemical grafting procedure and high chemical accessibility of bound molecules on top of grafted layers.

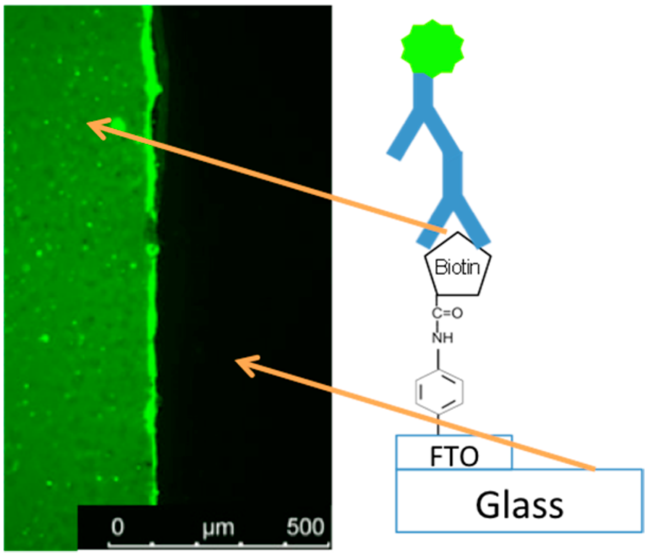


Figure 5. Immunofluorescence assay performed on grafted FTO. Fluorescence image (left) shows specific covalent modification of the surface during all procedure.

CONCLUSIONS

In this work patterned FTO samples were successfully selectively modified on the surface for the first time by means of electrochemical grafting of aryldiazonium cations produced in situ. Electrochemical measurements, XPS, and AFM outcomes provide quantitative information about the nature of the grafted film. Despite to other methodologies in literature concerning electrochemical grafting on other substrates, the surprising result is that a gentle modification is necessary to obtain a denser distribution of chemical sites on the surface. Also, chemical analysis of grafted film shows similarity to soluble aniline pK_a values confirming monolayer-like structure of the organic film: the possibility of controlling chemical functionalization of transparent conductive oxides (TCOs) is a most wanted perspective in optoelectronic based applications because it allows to modify bulk optoelectronic properties such as haze and band gap tuning. Also, biointegration of FTO electrodes is an attractive perspective: transparency is a fundamental request in biology, so the proof of concept of occurred biofunctionalization is provided.

ASSOCIATED CONTENT

Ⓢ Supporting Information

XPS and EIS characterization, estimates of RMS roughness, AFM topology images, and XPS comparison. This information is available free of charge via the Internet at http://pubs.acs.org/

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Author Contributions

F.L. performed all experiments. L.B. performed AFM measurements, and S.A. and G.G. performed XPS measurements and relative analysis. F.L., M.G., and N.E. designed the experiments and wrote the manuscript. M. G. and N. E. supervised the project.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

F. L. is supported by University of Padova grant. The author acknowledge funding from Italian Ministry for University and Research (MIUR). The authors thank Dr. Stefano Giulitti from the BioERA group of University of Padova for immunoassay test and fruitful discussion. Also, the authors thank Dr. Stefano Salmaso, PhD, of Department of Pharmaceutical and Pharmacological Sciences of University of Padova for interesting discussion about surface chemistry.

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