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# Physical and Electrochemical Properties of PEDOT:PSS as a Tool for Controlling Cell Growth

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## KEYWORDS

Bioelectronics, cell growth, conducting polymers, electrochemistry, redox state, surface properties.

## ABSTRACT.

Conducting polymers are promising materials for tissue engineering applications, since they can both provide a biocompatible scaffold for physical support of living cells, and transmit electrical and mechanical stimuli thanks to their electrical conductivity and reversible doping. In this work, thin films of one of the most promising materials for bioelectronics applications, poly(3,4-ethylenedioxythiophene) poly(styrenesulfonate) (PEDOT:PSS), are prepared using two different techniques, spin coating and electrochemical polymerization, and their oxidation state is subsequently changed electrochemically with the application of an external bias. The

electrochemical properties of these different types of PEDOT:PSS are studied through cyclic voltammetry and spectrophotometry to assess the effectiveness of the oxidation process and its stability over time. Their surface physical properties and their dependence on the redox state of PEDOT:PSS are investigated using atomic force microscopy (AFM), water contact angle goniometry and sheet resistance measurements. Finally, human glioblastoma multiforme cells (T98G) and primary human dermal fibroblasts (hDF) are cultured on PEDOT:PSS films with different oxidation states, finding that the effect of the substrate on the cell growth rate is strongly cell-dependent: T98G growth is enhanced by the reduced samples, while hDF growth is more effective only on the oxidized substrates that show a strong chemical interaction with the cell culture medium.

## 1. Introduction

Thanks to their favorable properties, namely chemical stability, low temperature processing, oxide-free surface in aqueous electrolyte, ionic and electronic transport, and mechanical compliance with living tissues, conjugated polymers (CPs), are promising materials for tissue engineering applications and are gaining more and more interest for the development of biosensors and bioactuators.<sup>1-3</sup> Among the many existing conducting polymers, poly(3,4-ethylene dioxythiophene) (PEDOT)-based materials, and especially its polystyrenesulfonate-doped (PEDOT:PSS) and p-toluenesulfonate-doped (PEDOT:TOS) forms, have become reference materials for the interfacing of electronics and living tissues. Over the last few years, many studies involving the effect of the oxidation state of CPs on cell adhesion, density and replication have been carried out.<sup>4-10</sup> In 1994, Wong *et al.* demonstrated that it is possible to control living cell growth through a change in the oxidation state of polypyrrole used as substrate for cell culture.<sup>4</sup> More recently, it has been shown that this effect involves a change in density

and conformation of adhesion proteins, which is strictly related to the oxidation state of the conducting polymer used as substrate.<sup>10,11</sup> It is known that cell-substrate interaction depends on the surface properties of the substrate itself: wettability,<sup>5</sup> surface roughness<sup>12-14</sup> and electrical conductivity<sup>15,16</sup> are key parameters in regulating this interaction, but their specific role in the adhesion process, together with the effect of electrochemical kinetics and protein-substrate interactions, are still not completely understood. For this reason, a systematic characterization of the surface properties of conjugated polymers, and more specifically on the dependency of these properties with respect to the oxidation state of conjugated polymers, would be of critical importance in order to disentangle the role of each different parameter involved in the cell-substrate adhesion process. However, a systematic approach to this problem is still lacking in literature. As can be seen from Table 1, where some of the main papers published in the last twenty years about the effect of the redox state of conjugated polymers on cell adhesion and growth are summarized, each study is carried out in different experimental conditions, in terms of material used, oxidation process, cell line observed and cell growth conditions. Such variability in sample preparation and biological conditions brought about the observed very scattered results, all of them confirming that it is possible to control cell adhesion and growth, but hindering the possibility to draw general conclusions. Moreover, although the electrochemical properties of conductive polymers are exploited in several applications, such as electrochemical transistors,<sup>17</sup> actuators,<sup>18</sup> electrochromic devices,<sup>19</sup> smart membranes and drug delivery devices,<sup>20</sup> the physical and chemical processes involved in the doping/dedoping reactions have not yet been clarified. The aim of the present work is to assess the effects induced by a change in the redox state of PEDOT:PSS on the main physical parameters which are related to the cell adhesion process, which we identified as surface morphology, electrical conductivity,

wettability and oxidation state, and to show that a precise control of these conditions is possible and necessary to achieve reproducible results. We therefore carried out cell growth experiments on four different types of PEDOT:PSS using two different cell lines, primary human dermal fibroblasts (hDF) and human glioblastoma multiforme cells (T98G). These types of cells have been chosen in order to assess the effects of the substrates parameters on adherent, high-replicating and tissue-forming cells with very similar genotypic and phenotypic properties but normal (hDF) and tumor-like (T98G).

## 2. Experimental Section

*Materials:* Clevios CPP105D and Clevios PH1000 were purchased from Heraeus. Ethylene glycol, dodecyl benzene sulfonic acid (DBSA), 3-glycidoxypropyl trimethoxysilane (GOPS), EDOT monomer, PSS and phosphate buffer saline solution (PBS) were purchased from Sigma-Aldrich.

*Fabrication of the PEDOT:PSS substrates:* Two different formulations of PEDOT:PSS were used, Clevios CPP105D and Clevios PH1000. The former is a low conductive formulation, which is optimized to be deposited on hydrophobic substrates, while the latter is optimized to be highly conductive and was mixed with ethylene glycol (20 v/v %) and DBSA (0.05 v/v %) in order to improve its electrical conductivity and the ease of the spin coating deposition. A cross-linking agent, GOPS 1 v/v %, was added to both CPP105D and PH1000 in order to improve their resistance to delamination when exposed to water. These two suspensions were treated in ultrasonic bath for 10 minutes and filtered using 1.2  $\mu\text{m}$  cellulose acetate filters (Sartorius), then spin coated over clean glass slides at 1000 rpm for 10 s. The samples were subsequently dried at 140  $^{\circ}\text{C}$  for 30 minutes to remove water and other solvents.

Reference	Polymer <sup>a)</sup>	Cell line	Applied potential (electrolyte)	Time of biasing	Serum <sup>b)</sup>	Preferred side
Wong 1994 <sup>4</sup>	Ppy Fn coating	Bovine aortic endothelial cells	0 / -0.25 V (growth medium)	During growth	FBS	Oxidized
					Serum free	Oxidized
Saltó 2008 <sup>5</sup>	PEDOT:TOS	Immortalized mouse neural stem cells	± 0.75 V (NaCl)	During growth	Serum free	Oxidized
		Non-immortalized mouse neural cells				Oxidized
Svennersten 2009 <sup>6</sup>	PEDOT:TOS	Canine kidney epithelial cells	± 0.75 V (growth medium)	During growth	FBS	Reduced
					Serum free	Both
	PEDOT:TOS Fn coating				FBS	Reduced
					Serum free	Reduced
Wan 2009 <sup>7</sup>	PEDOT:TOS	Mouse fibroblasts	± 1 V (growth medium)	1 h Before seeding	FBS	Oxidized
		Human breast cancer cells				Oxidized
Greco 2013 <sup>8</sup>	PEDOT:PSS Fn coating	Murine skeletal muscle cells	± 0.75 V (growth medium)	During growth	FBS	Reduced
		Normal human dermal fibroblasts				Reduced
Sivaraman 2013 <sup>9</sup>	Ppy	Mouse embryonic fibroblasts	Cyclic +0.4 ÷ +1 V / 0 ÷ -0.7 V (NaDBS <sup>c)</sup> )	10 min Before seeding	FBS	Oxidized
	Ppy-PEG					Oxidized
Wan 2012 <sup>10</sup>	PEDOT:TOS	Mouse fibroblasts	± 1 V (growth medium)	1 h Before seeding	Serum free	Oxidized

<sup>a)</sup> Fn: fibronectin; PEG: polyethylene glycol; <sup>b)</sup> FBS: fetal bovine serum; <sup>c)</sup> NaDBS: Sodium dodecylbenzenesulfonate.

**Table 1.** Comparison between literature results on cell adhesion on conducting polymers and their experimental conditions.

Electropolymerized PEDOT:PSS was synthesized onto a previous deposited PEDOT:PSS (CPP105D) thin film prepared as described above, with the only exception that no GOPS was added to the suspension, since this addition increased dramatically the electrical resistance of the conducting polymer. In order to increase the electrical conductivity, the coated glass slide was dipped in ethylene glycol and was dried again at 140°C for 40 min. The electrodeposition was carried out by cyclic voltammetry (CV) ( $E_{low} = 0$  V;  $E_{high} = +1.5$  V; scan rate =  $0.1 \text{ V s}^{-1}$ ) employing a three electrode cell equipped with a Saturated Calomel Electrode (SCE) as reference electrode, and a Pt wire as counter electrode. The PEDOT:PSS spin coated film acted as working electrode, after ensuring the electrical contact to the potentiostat with conductive silver paint. The electropolymerization was carried out by dipping the electrodes in a deaerated solution containing 10 mM EDOT and 0.1 mM PSS. Two different film thicknesses were obtained by employing 4 or 8 deposition cycles (ED4 and ED8, respectively). CPP105D was chosen as a substrate for electrochemical polymerization, instead of the metals commonly used for this purpose, such as gold or ITO, so as to obtain transparent and all-organic films which can be employed in low-cost and flexible devices. Moreover, a decrease in the delamination of electropolymerized PEDOT:PSS was observed when deposited on CPP105D-coated glass slides instead of metal electrodes, due to the good adhesion of CPP105D to glass.

The oxidation state of the PEDOT:PSS films was changed using the three-electrode cell setup described in the previous paragraph. The PEDOT:PSS films were connected to the potentiostat and used as working electrodes in PBS. The samples were kept at a continuous bias *vs.* SCE for 1 h in order to obtain an oxidized (positive voltage applied) or reduced (negative voltage applied) form of PEDOT:PSS. This time interval was chosen after analyzing the trend of current *vs.* time during polarization, so as to ensure a complete and stable polarization. The samples were then



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3 rinsed in distilled water and dried at room temperature. It must be noted that the use of a  
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5 reference electrode during the biasing of the samples was proven to be essential to achieve a  
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7 reproducible oxidation state of PEDOT:PSS, since without this reference, the actual chemical  
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9 potential of the polymer films is basically random. Different biases were used to study the  
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11 voltage dependence of the physical and chemical properties of this polymer, and their different  
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13 effect on cell replication. In the present work, only data acquired from the samples treated with  
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15 the highest potentials we tested,  $V = +0.8$  V (oxidized) and  $V = -0.9$  V (reduced), are reported,  
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17 since they show the most significant effect.  
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22 *Electrochemical characterization:* All the electrochemical experiments were carried out in a  
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24 three electrode cell equipped with a SCE as reference electrode and a Pt wire as counter  
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26 electrode. The responses were recorded using PGSTAT20 (Ecochemie) connected to a personal  
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28 computer. The electrochemical characterizations were performed by cyclic voltammetry between  
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30 -1.6 V and +0.8 V employing a scan rate of  $20 \text{ mV s}^{-1}$ . The samples characterized by CV were  
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32 used only for such experiments, and not for any further characterization. The stability of the  
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34 electrochemically-generated redox states were evaluated by spectroelectrochemical experiments  
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36 using a HewlettPackard 8453 diode array spectrophotometer. The samples were biased for 1h by  
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38 applying a continuous voltage in PBS, and subsequently Vis-NIR (300-1000 nm) spectra were  
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40 collected for 60 min every 1 min after the end of the polarization.  
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46 *Atomic force microscopy:* Imaging of the surface of the PEDOT:PSS films was carried out  
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48 using a Park NX10 atomic force microscope (AFM) operating in non-contact mode in air in  
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50 ambient conditions. Root mean square roughness ( $R_q$ ) and thickness ( $t$ ) of the polymer films  
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52 were evaluated through the acquisition of topographical images with scan size  $5 \mu\text{m} \times 5 \mu\text{m}$ . In  
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order to assess the homogeneity of the electrodeposition process, scans were performed at three different positions on each film for several samples.

*Wettability measurements:* Hydrophilicity and surface energy of the PEDOT:PSS films were determined by water contact angle measurements (surface tension of  $72.8 \text{ mN m}^{-1}$ ), which were performed with a custom setup using the sessile drop technique. The average contact angle value was obtained by measuring the contact angles at two different positions on two different samples, and the error on the average was evaluated as the standard deviation of this set of measurements for each material. The contact angles were converted into surface energy values employing the relation of E. Chibowski *et al.*<sup>21</sup>

*Surface resistivity measurements:* The sheet resistance ( $R_s$ ) of the polymer films was measured using a 4-probe custom setup, where four aligned and evenly spaced (spacing 2.7 mm) conductive tips were connected to a Keithley 2400 SourceMeter, which was used as a current generator for the two external tips and as a voltage meter for the two inner tips. Sheet resistance was measured at three different positions for each sample on at least three samples. An ideal geometrical correction factor of  $\pi/\ln 2$  was applied to all measurements.<sup>22</sup> The reported error on the average was evaluated as the standard deviation of the set of measurements carried out for each material.

*Cell culture:* Glioblastoma multiforme cells (T98G), derived from a human tumor, were maintained in Roswell Park Memorial Institute (RPMI) medium, supplemented with 10% fetal bovine serum, 1% L-glutamine, 10% sodium pyruvate and antibiotics (1% penicillin and 1% streptomycin). Primary dermal fibroblast (hDF), gently provided by Prof. S. Salvioli (DIMES, University of Bologna), were obtained from two donors (age 24 and 23 years) by skin biopsies according to standard culture method. All the donors gave their informed consent before biopsy

was performed. hDF were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum, 1% L-glutamine and antibiotics (1% penicillin and 1% streptomycin). hDF of 3-15 passages number were used. Both cells population were kept in culture standard conditions in incubator at 5% CO<sub>2</sub> humidified atmosphere at 37°C. Before experiment cells were detached by trypsin (0.02%) in ethylenediaminetetraacetic acid (EDTA) and re-suspended in culture medium at room temperature (all reagents were purchased from Sigma Chemical Italy, Milano, Italy)

*Proliferation curves:* the cell culture 24-wells plates (CELLSTAR, Greiner bio-one) were prepared as follow: PEDOT:PSS substrates in its non-biased, oxidized and reduced form were cut using a diamond tip and three replication for each form were placed in nine different wells of the plate; the plate were sterilized under UV per 20 min. Before cells seeding the PEDOT:PSS was washed out with sterile culture medium in order to buffer the pH altered by the substrates.

T98G and hDF cells were counted by an hemocytometer and seeded in a density of  $0.010 \times 10^6$  and  $0.015 \times 10^6$  with 500 µl of supplemented RPMI and DMEM, respectively, in each well prepared as described and in three wells without PEDOT:PSS, i.e. on a sterile hydrophilic polystyrene tissue culture surface (control) physically treated for improving cell adhesion. Cells were allowed to adhere and spread for 24 h prior the observation.

We studied cell proliferation by growing T98G and hDF in a CO<sub>2</sub> incubation system integrated within a motorized stage allowed to perform time-lapse imaging acquisition even for tens of hours. Images were acquired in phase-contrast at 100× of magnification for a time interval up to 168 h (seven days) by the inverse automatized optical microscopy Nikon Eclipse-Ti (Nikon, Italy).

### 3. Results and Discussion

Two different formulations of the same material, PEDOT:PSS, were employed to fabricate thin films using two techniques, spin coating and electrochemical polymerization, obtaining four different types of samples, listed in Table 2. Clevios CPP105D and Clevios PH1000 are commercial formulations deposited on glass by spin coating, while ED4 and ED8 refer to electrochemically deposited PEDOT:PSS obtained by applying, respectively, 4 and 8 polymerization cycles (the details on their preparation are reported in the Experimental Section) to CPP105D-coated glass slides. These films were characterized in terms of thickness, surface morphology, wettability and electrical resistance, and they showed quite different properties. Since cell growth experiments require long-term interaction (usually days, or even weeks) between the physical substrate of the cells and an aqueous electrolyte solution, the assessment of the chemical and electrochemical kinetics of the substrate itself is crucial. For this reason, the stability of our PEDOT:PSS films was studied through cyclic voltammetry and spectrophotometry. These measurements were carried out in different aqueous media and for different redox voltages, namely between  $\pm 0.3$  V and  $\pm 0.9$  V vs. Saturated Calomel Electrode (SCE), to identify the experimental conditions that optimize the effectiveness and the stability of the electrochemical modification of the oxidation state of PEDOT:PSS. The highest potential difference,  $+0.8$  V /  $-0.9$  V, was chosen in order to maximize the differences between the physical properties of oxidized, not biased and reduced state of PEDOT:PSS (see Supporting Information for electrical surface resistance).

Name	Deposition technique	Substrate	Active sites concentration (mol dm <sup>-3</sup> ) <sup>a)</sup>
CPP105D	Spin coating	Glass	0.21 ± 0.06
ED4	Electrochemical polymerization (potentiodynamic, 4 cycles)	Glass + CPP105D	0.54 ± 0.16
ED8	Electrochemical polymerization (potentiodynamic, 8 cycles)	Glass + CPP105D	0.55 ± 0.14
PH1000	Spin coating	Glass	0.16 ± 0.02

<sup>a)</sup> Calculated from cyclic voltammetry

**Table 2.** Different types of PEDOT:PSS used in this work.

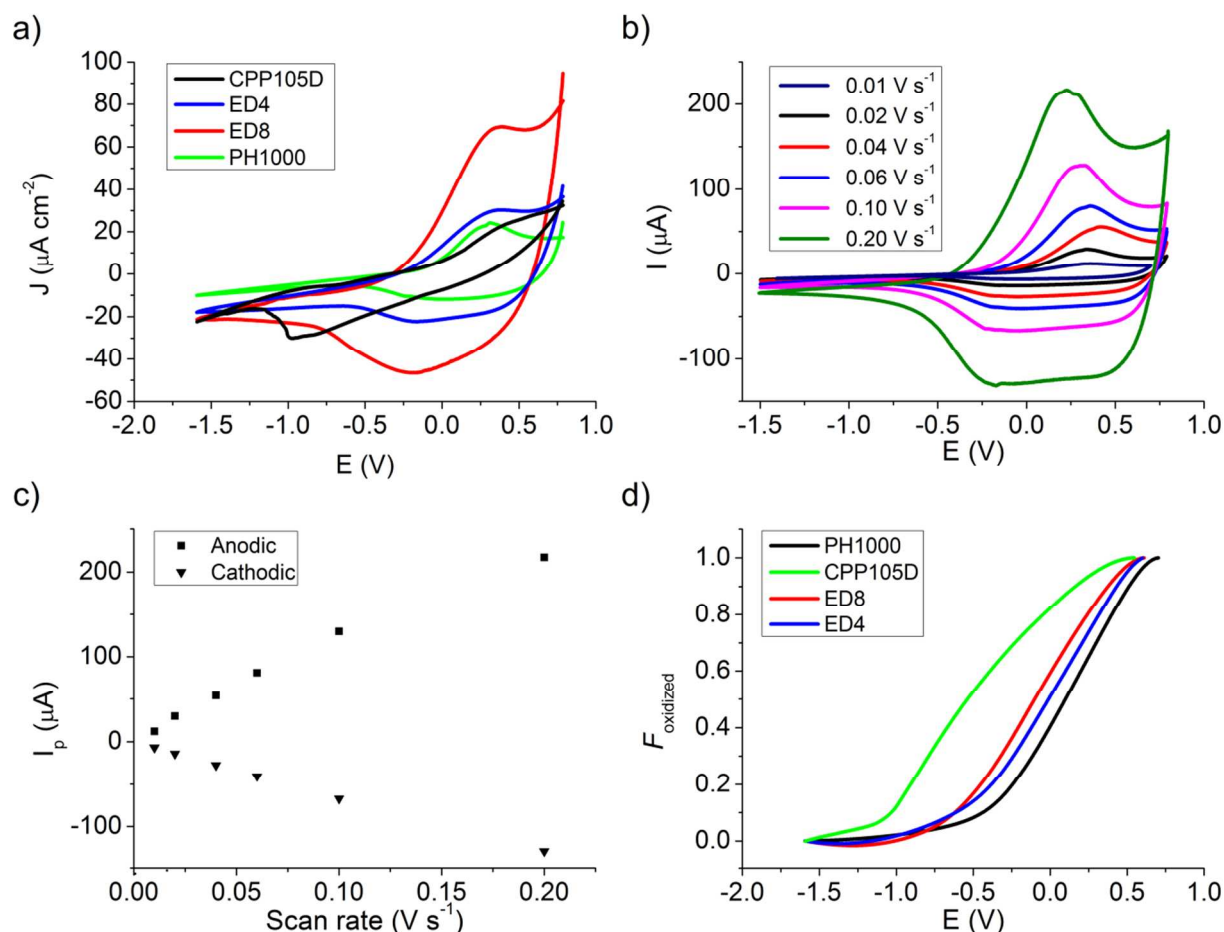
**3.1 Electrochemical characterization**

The electrochemical properties of PEDOT:PSS samples were studied by cyclic voltammetry (CV) with the same geometry used for cell growth, without employing a supporting current collector. The CV responses are very stable between -0.2 and +0.6 V. Nevertheless, it was possible to carry out a reliable electrochemical characterization using a wider range of the applied potential, up to the maximum values achievable before an irreversible degradation process set in. The CVs (Figure 1a) which were obtained for the four types of PEDOT:PSS samples under examination display a couple of peaks that are well-defined for PH1000, ED4 and ED8, whereas the redox waves of CPP105D are less defined and their baseline is tilted, probably due to a higher electrical resistance (see Paragraph 3.3).

The peak currents are directly proportional to the scan rate in the range 0.010 ÷ 0.100 V s<sup>-1</sup> (Figure 1b and 1c show the results for PH1000, see Supporting Information for the others), highlighting that diffusion is not the limiting step of the electrochemical processes and thus that the whole film thickness is involved in the electrochemical reaction.<sup>23</sup> Therefore, the charge

flowing during the CV scans corresponds to the electrons that are needed to change the redox state of the whole PEDOT:PSS volume. Consequently, the degree of electrochemical doping can be calculated for the different materials per each potential. From Figure 1d, which reports the fraction of oxidized PEDOT sites over the number of total active sites (i.e. where electrons are exchanged), it is possible to see that the active sites that take part in the redox processes are almost completely reduced ( $F_{oxidized} \approx 0$ ) at -0.9 V. On the other hand, the anodic limit for polarization is found at +0.8 V, since it was observed that higher potential values lead to a strong overoxidation of the conductive polymer.

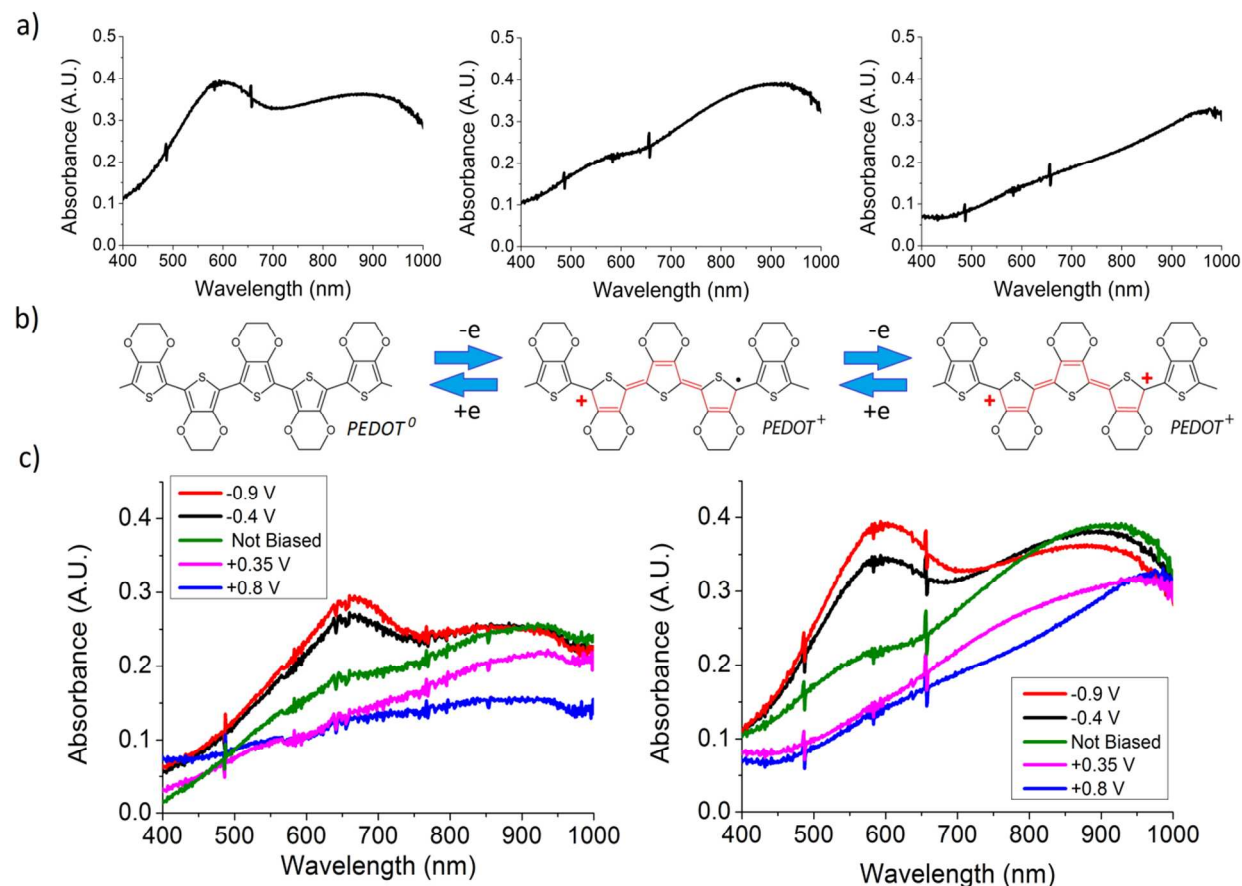
If we assume that only one charge interacts with each active site of the conductive polymer, it is also possible to calculate the concentration of the sites that change their redox state by measuring the charge that flows during the cathodic scan of a cyclic voltammetry. This concentration, that can be directly correlated to the number of charge carriers, resulted equal to 0.55, 0.54, 0.21, 0.16 mol dm<sup>-3</sup> for ED4, ED8, CPP105D and PH1000, respectively (Table 2). The larger values of electrochemically polymerized PEDOT:PSS can be ascribed to its higher PEDOT/PSS ratio.<sup>24</sup>



**Figure 1.** a) CVs recorded for different PEDOT:PSS substrates (scan rate 20 mV s<sup>-1</sup>). b) Cyclic voltammograms of PH1000 taken at different scan rates. c) Peak current vs. scan rate. d) Fraction of oxidized PEDOT:PSS ( $F_{\text{oxidized}}$ ) for the different substrates as a function of the applied voltage. All the graphs refer to measurements carried out in PBS.

Although only one wave is observed both in the cathodic and anodic scan, many redox processes occur during PEDOT:PSS reduction/oxidation.<sup>25</sup> The existence of at least three redox states of PEDOT:PSS can be seen in the visible and near infrared (NIR) spectra recorded on the films biased at different potentials (Figure 2). The spectrum of native PEDOT:PSS displays a large band at about 900 nm that is ascribable to polaron absorption.<sup>26</sup> The spectrum recorded

when PEDOT:PSS was biased at +0.8 V does not show any peak at 900 nm, since such potential is sufficient to oxidize polarons, forming bipolarons and turning PEDOT:PSS into its highly conductive form (Figure 2b), that is almost transparent.<sup>27</sup>



**Figure 2.** a) Visible and NIR spectra of ED8 in its reduced (-0.9 V, left), not-biased (middle) and oxidized (+0.8 V, right) forms. b) Schematics of the redox process in PEDOT from its reduced (left) to its fully oxidized (right) state. c) Visible and near IR spectra of PH1000 (left) and ED8 (right) in their native state and biased at -0.9 V, -0.4 V, +0.35 V and +0.8 V.

On the other hand, the spectrum recorded for PEDOT:PSS biased at +0.35 V displays a shoulder at about 800 nm, suggesting that the polarons are not completely oxidized in bipolarons. The spectrum recorded when PEDOT:PSS was biased at -0.9 V shows two peaks. The first one,



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3 at about 600 nm, is assigned to the transition  $\pi \rightarrow \pi^*$  of the polymer in its neutral state,  
4 highlighting the reduction of the polarons caused by the injection of electrons into the material,  
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6 as shown in Figure 2b. However, the band at 900 nm points out that charged polarons are still  
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8 present in PEDOT:PSS, suggesting that the reduction of this material is incomplete at -0.9 V.  
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10 This effect was explained in literature with the presence of an aqueous medium, which slowly  
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12 oxidizes PEDOT.<sup>28</sup> Finally, the spectrum recorded at -0.4 V also shows a peak at 600 nm (Figure  
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14 2c), but its intensity is slightly lower with respect to the spectrum obtained at -0.9 V, meaning  
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16 that a lower percentage of PEDOT is in its neutral form.  
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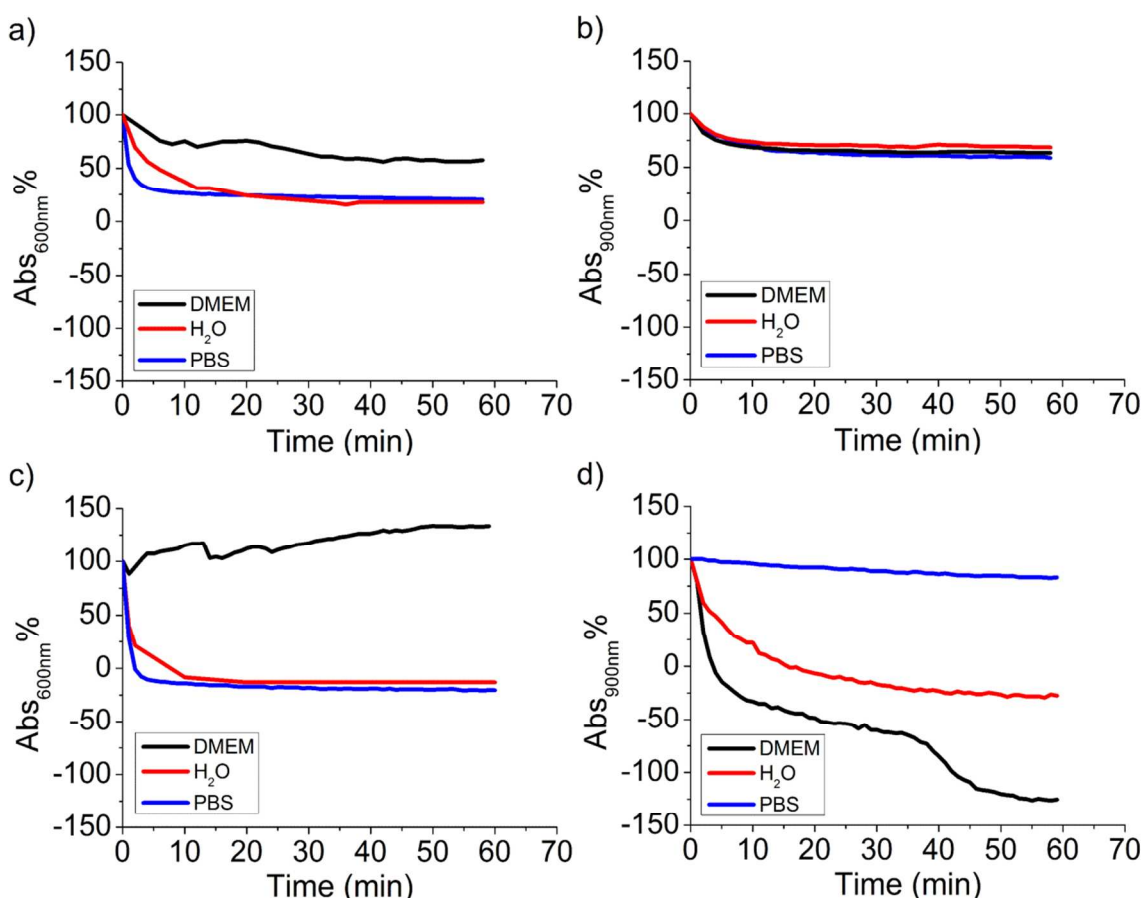
22 Having assessed the redox effects induced on PEDOT:PSS thin films, we prepared  
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24 PEDOT:PSS substrates for our cell growth experiments by choosing three applied bias values  
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26 that generate three different sets of samples: one containing mainly neutral PEDOT, one  
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28 containing mainly polarons and one wherein PEDOT is present in its high conductive state. In  
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30 order to maximize the difference in performance of these three types of substrates, PEDOT:PSS  
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32 was thus used either in its pristine state or biased at -0.9 and +0.8 V.  
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36 The stability in time of the electrochemically generated redox state of PEDOT:PSS thin films  
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38 plays a key role in their effective use as cell growth substrates. To ensure long-term stability of  
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40 the redox state, the films were biased for 1 h and we thus monitored the modifications that  
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42 occurred after the applied bias was removed, by collecting the visible and NIR spectra of  
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44 reduced/oxidized PEDOT:PSS while immersed in three different aqueous media: distilled water  
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46 (H<sub>2</sub>O), phosphate buffer solution (PBS) and Dulbecco's modified Eagle's medium (DMEM)  
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48 supplemented with fetal bovine serum. These media were used because of their different  
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50 composition, since PBS contains small ions only while DMEM also contains quite large organic  
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52 molecules, like amino acids, vitamins and glucose. The stability of the induced redox state was  
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continuously monitored for one hour after removing the applied bias (a spectrum was acquired every 2 minutes) and we observed how the PEDOT:PSS spectrum changed, reaching a stable condition after 20 min. The spectra collected for oxidized and reduced ED4 are presented in the Supporting Information.

Figure 3 reports the evolution in time of the quantity  $Abs_{600nm}\%$ , that is calculated as:

$$Abs_{600nm}\%(t) = \frac{A_{600nm}(t) - A_{600nm\ native}}{A_{600nm}(0) - A_{600nm\ native}} \times 100$$



**Figure 3.** Trends of  $Abs_{600nm}\%$  and  $Abs_{900nm}\%$  for (a) reduced ED4, (b) oxidized ED4, (c) reduced PH1000, and (d) oxidized PH1000. The samples were biased at -0.9 and +0.8 V, and then immersed in DMEM, H<sub>2</sub>O and PBS. The trends for CPP105D and ED8 are available in the Supporting Information.

Where  $A_{600nm}(0)$  and  $A_{600nm}(t)$  are, respectively, the absorbance at 600 nm of biased PEDOT:PSS just after it was soaked in the medium and after being dipped for a time  $t$ , while  $A_{600nm native}$  is the absorbance at 600 nm of native PEDOT:PSS. Analogously, the quantity  $Abs_{900nm}\%$ , also reported in Figure 3, refers to the absorbance peak at 900 nm.

The spectrum of negative-biased substrates displays a decrease of the absorbance peak at 600 nm that is due to the transition  $\pi \rightarrow \pi^*$  of the neutral form of PEDOT generated by the polarization at -0.9 V. Therefore, the amount of neutral PEDOT that is still present in the sample at the time  $t$  is proportional to  $Abs_{600nm}\%$ .

When PEDOT:PSS is biased at +0.8 V, an increase in the peak at about 900 nm is observed after the applied bias is removed, due the formation of polarons or bipolarons (Figure 2). Therefore, the amount of highly conductive PEDOT:PSS that has turned in the average oxidized state can be estimated by the recovery of absorbance at 900 nm,  $Abs_{900nm}\%$ .

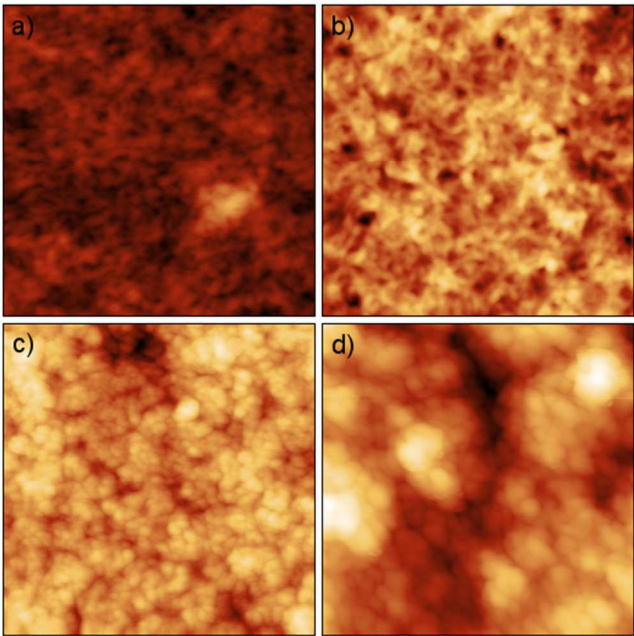
Figure 3 shows that the reduced forms of PEDOT:PSS revert to their pristine state in a few minutes when exposed to distilled water and PBS, while in DMEM their absorbance drops at about 60% of its starting value and then stabilizes. The oxidized forms are more stable in time than the reduced ones for any tested medium for substrates ED4 (Figure 3a, b), ED8 and CPP105D (Supporting Information), as suggested by preliminary studies in water.<sup>28,29</sup> After one hour, the residual oxidation of all the samples is about 60% of the initial state, with the only exception of PH1000 (Figure 3c, d). DMEM acted strongly on the redox state of oxidized PH1000, leading to the reduction of PEDOT. The same measurement in DMEM was repeated by monitoring the PEDOT:PSS spectrum for 48 h, in order to assess its stability in cell culture medium for a time period comparable to the duration of biological experiments (Supporting information). As a result, the difference in the absorption spectra of oxidized and reduced

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3 samples was still observable, with a residual oxidation of about 90% of the starting value for the  
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5 oxidized samples and about 30% for the reduced ones, attesting the effectiveness of the redox  
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7 process. Moreover, the spectrum of oxidized PH1000 showed an absorbance peak between 300  
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9 nm and 400 nm after 48 h of immersion in DMEM which was not present at the beginning of the  
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11 measurement, suggesting the occurrence of a chemical modification induced by the cell culture  
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13 medium.  
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17 From these observations we can conclude that, although the generated redox states are not  
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19 completely stable in time, a sharp difference between the oxidized and reduced films is still  
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21 present after the removal of the redox voltage for all 4 types of PEDOT:PSS samples here  
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23 investigated, meaning that the application of a continuous bias during cell growth is not  
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25 necessary to grant an effective difference in the PEDOT:PSS substrate redox state.  
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### 28 29 **3.2 Surface roughness and wettability**

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31 AFM imaging was used to evaluate the surface roughness and the thickness of thin films of  
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33 different types of PEDOT:PSS. Topography maps of the different samples are shown in Figure 4,  
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35 while the corresponding roughness and thickness values are reported in Table 3, together with  
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37 surface energy of oxidized and reduced PEDOT:PSS calculated numerically from water contact  
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39 angle measurements taken before and after the electrochemical redox process, as described  
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41 below in this paragraph.  
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**Figure 4.** AFM topography images of the PEDOT:PSS samples under study: a) PH1000, b) CPP105D, c) ED4, d) ED8. Scan size: 1  $\mu\text{m}$  x 1  $\mu\text{m}$ , z scale: 35 nm for (a), (b) and (c), 90 nm for (d).

Redox voltage (V)	RMS Roughness (nm)	Thickness (nm)	Surface Energy ( $\text{mJ m}^{-2}$ )			
			-0.9	0	0 (after 1h in PBS)	+0.8
CPP105D	$6 \pm 1$	$260 \pm 70$	$43 \pm 3$	$68 \pm 1$	$47 \pm 2$	$41 \pm 3$
ED4	$22 \pm 4$	$240 \pm 80$	$56 \pm 3$	$68 \pm 2$	$51 \pm 2$	$50 \pm 1$
ED8	$33 \pm 2$	$480 \pm 120$	$56 \pm 2$	$70 \pm 1$	$57 \pm 3$	$54 \pm 2$
PH1000	$13 \pm 3$	$440 \pm 30$	$45 \pm 4$	$57 \pm 3$	$47 \pm 3$	$51 \pm 2$

**Table 3.** RMS roughness, thickness and surface energy of the samples under study.

Root mean square (RMS) roughness,  $R_q$ , was evaluated from topography images with a scan area of 20  $\mu\text{m}$  x 20  $\mu\text{m}$ . CPP105D has the flattest surface ( $R_q$  of about 6 nm), while ED8 shows the highest roughness ( $R_q$  about 33 nm). The comparison between CPP105D, ED4 and ED8 shows that it is possible to increase the surface roughness of PEDOT:PSS by controlling the number of polymerization cycles. This increase in surface roughness is due to the formation of

PEDOT-rich globular structures of increasing size, as can be seen from Figure 4. The thickness of the samples is basically not affected by the first four cycles of polymerization, while the following four cycles increase the thickness of PEDOT:PSS by about 100% of its starting value. This effect can be explained by assuming that polymerization starts inside the volume of CPP105D, used as conductive electrode, during the first cycles of this process, and then the electropolymerized PEDOT:PSS starts growing on the surface of the electrode only after a certain number of cycles, increasing the overall film thickness. Finally, we preliminarily assessed the effect induced by the redox process on the surface morphology of the PEDOT:PSS thin films here investigated, finding no significant variation from the roughness of pristine substrates, even for the highest biases used, -0.9 V and +0.8 V (data not shown).

Water contact angle measurements were carried out on the samples just after deposition (pristine) and after 1 h of polarization in PBS, by applying the same potentials used in cell growth experiments. As a control, some of the samples were immersed in PBS for 1 h with no bias applied. The results are reported in Figure 5a. All the samples are hydrophilic (contact angle is always below 90°). However, regarding the pristine form of PEDOT:PSS, there is a clear difference between CPP105D-based films (both ED4 and ED8 are polymerized using CPP105D films as working electrode) and PH1000, which shows a lower hydrophilicity. The comparison between the contact angles measured before and after the exposure to PBS clearly points out that a decrease in the surface energy of PEDOT:PSS is induced by the interaction with the electrolyte solution, irrespectively to the applied voltage (Table 3). This effect can be explained by considering that PSS in excess is partially removed when the films are immersed in water.<sup>30</sup> Excess PSS is expected to be mostly accumulated on the surface of the films,<sup>31</sup> and because of the presence of  $\text{HSO}_3^-$  groups, it can be dissolved by water thanks to the formation of hydrogen

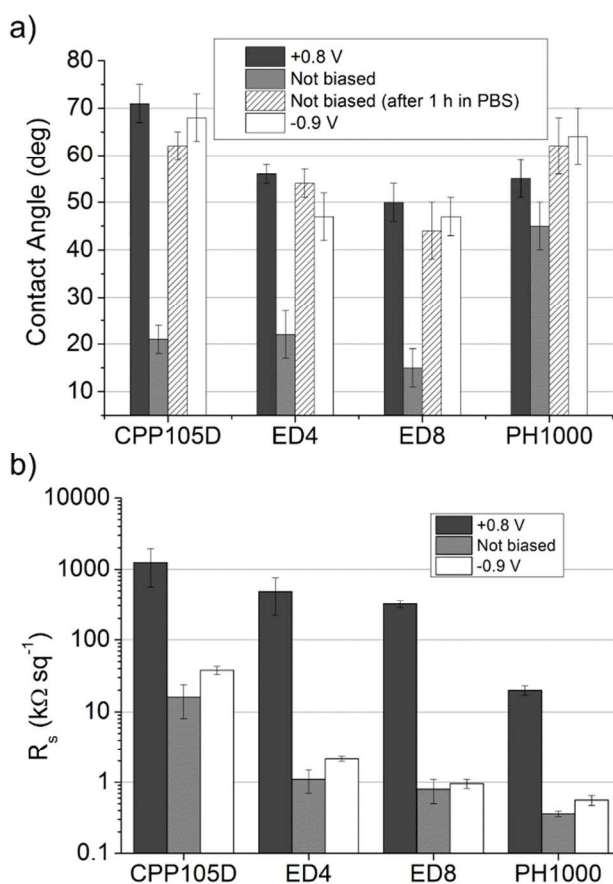
bonds, making the surface of the film hydrophilic. Since the PSS in excess is poorly interacting with PEDOT, its removal in water has no significant effect on the electrical and electrochemical properties of PEDOT:PSS. On the other hand, a change in the oxidation state of PEDOT:PSS does not seem to influence the hydrophilicity and the surface energy of this material, conversely to what was observed for instance on PEDOT:Tosylate.<sup>5</sup> Thanks to their relatively small dimensions compared to PSS (171 vs. 70,000 Da), tosylate anions can be completely removed from the polymer matrix and undergo ion exchange when immersed in an electrolyte solution,<sup>32</sup> while this effect is hindered for PSS due to steric effects. As a consequence, the exchange of PSS anions is mechanically impeded, as well as the switching of charged sulfonate groups from PEDOT to the surface. This explanation is confirmed by the fact that PEDOT:PSS exchanges cations ( $\text{Na}^+$ ,  $\text{K}^+$ , etc.) with the electrolyte solution to maintain electrical neutrality,<sup>33</sup> while the polymer exchanges its counterion when small dopant molecules, like tosylate or perchlorate, are used.<sup>32</sup> Moreover, since the outer part of the film is mainly composed by an excess of PSS, the variation of the redox state of inner PEDOT does not affect the surface processes. As a result, the surface energy of the films is weakly influenced, if not at all, by the applied polarization, even if charge distribution in the PEDOT cores is effectively modified.

These results suggest that, regarding PEDOT:PSS, surface energy and wettability cannot be considered key parameters for controlling cell growth.

### 3.3 Sheet resistance

The sheet resistance ( $R_s$ ) of PEDOT:PSS thin films was measured using a 4-probe setup before and after the application of a redox voltage to the samples for 1 h. The results are reported in Figure 5b. PH1000 films have the lowest sheet resistance ( $R_s$  about 0.36 k $\Omega$ /sq). ED4 and ED8 show slightly higher values ( $R_s$  about 1.1 and 0.8 k $\Omega$ /sq, respectively), but still comparable,

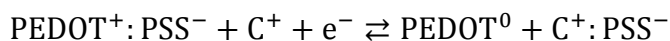
while the sheet resistance of CPP105D is at least ten times higher compared to the other materials. This high difference is partially due to the absence of the cross-linking agent (GOPS), which is proven to affect PEDOT:PSS conductivity,<sup>30</sup> in the CPP105D suspension used as substrate for electropolymerization. Indeed, the presence of GOPS 1 v/v% was found to increase the surface resistance of CPP105D and PH1000 by a factor of 2.



**Figure 5.** a) Water static contact angle measured on the PEDOT:PSS samples under study in their oxidized, not biased and reduced form. The not biased samples were measured as deposited and after being immersed in PBS for 1 h. b) Sheet resistance measured on all the four different sets of PEDOT:PSS samples here investigated, in their oxidized, not biased and reduced form.



The effect of the electrochemical redox reaction on the conductivity of PEDOT:PSS depends on the polarization (anodic or cathodic) of the voltage applied to the electrode. When a reducing (i.e. negative) potential is applied to the PEDOT:PSS electrode, the following reaction occurs:<sup>33</sup>



Where  $\text{C}^+$  and  $\text{e}^-$  are, respectively, cations and electrons within the electrolyte solution. Hence, PEDOT changes from its oxidized (conductive) state to its neutral (not conductive) state, and an increase in the sheet resistance is observed. When an oxidizing (i.e. positive) voltage is applied to the PEDOT:PSS electrode, the polymer film changes from its pristine partially-oxidized state to a more conductive fully-oxidized state. However, if the positive bias is over 0.6 V, PEDOT:PSS undergoes overoxidation, which induces a dramatic increase in electrical resistance, as observed in all our tested samples. It is noteworthy that, despite the higher carrier density measured in the electrodeposited samples (see Paragraph 3.1), PH1000 shows the highest electrical conductivity, indicating that in PH1000 the charge carriers have a much larger mobility that compensates their lower density. Finally, the effect of the exposure to cell culture medium (DMEM) was tested on not biased samples for up to 48 h, finding that the surface resistance increases by about a factor of two in this time period (see Supporting Information). This change mostly takes place within the first hour, indicating that is probably due to a spontaneous reorganization of the polymer chains induced by the interaction with the electrolyte (which lowers carrier mobility) rather than a degradation effect.

By selecting the redox potentials reported in Figure 5b we can thus obtain PEDOT:PSS thin films with different yet controlled electrical transport properties, one of the key parameters whose role has to be assessed when PEDOT:PSS is used as a cell growth substrate.

### 3.4 Cell growth

In order to assess the viability and the different efficacy of PEDOT:PSS thin films as substrates for controlled tissue growth applications, the proliferation rate of two different cell lines, human glioblastoma multiforme cells (T98G) and primary human dermal fibroblasts (hDF), was tested on the polystyrene substrate of cell culture plates (control), on the four types of pristine PEDOT:PSS substrates (not biased) or prepared by applying a redox voltage of +0.8 V (oxidizing) or -0.9 V (reducing). The samples were biased for 1 h in PBS and then disconnected from the generator prior to cell seeding. These values for the applied redox potential were chosen since they induced the most significant difference in PEDOT:PSS physical properties (see previous paragraphs). The proliferation curves of both cells population have been obtained by normalizing the mean number of adherent cells counted from fixed focal fields (set when the first image section has been acquired, i.e. 24 h from seeding) after 24 h, 48 h and 72 h (see Experimental section). The statistical comparison between cell growth on the different not biased PEDOT:PSS substrates and on the polystyrene wells surface (control) is presented in the Supporting Information. The obtained results can be summarized as follow:

- The normal cells (hDF) growth rate measured at 72 h from seeding on each type of PEDOT:PSS tested is not significantly different compared the others (not biased, reduced and oxidized), with the only exception of oxidized (+0.8 V) PH1000, where a significant increase in hDF growth compared to the not biased sample was observed (Figure 6a and 7).
- The tumor cells (T98G) growth rate showed a significant increase on all the tested substrates that had been reduced (-0.9 V). Indeed, the statistical analysis highlighted that, at 72 h from seeding on all the four types of reduced PEDOT:PSS substrates, the cell proliferation rate is significantly enhanced compared to the not biased and oxidized ones,

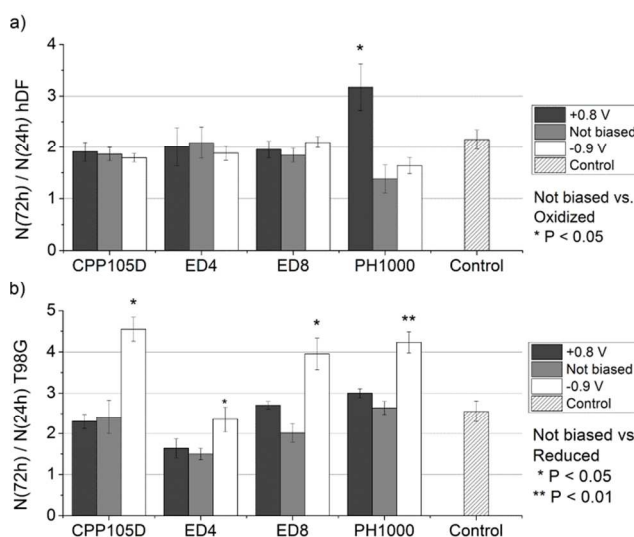
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3 irrespective of the type of film used (Figure 6b and 7). Furthermore, within the  
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5 experimental errors, the T98G proliferation rate is the same on all the not biased  
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7 PEDOT:PSS substrates, with the only exception of ED4.  
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10 As indicated by the physical parameters of PEDOT:PSS, the techniques used for the film  
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12 depositions create surfaces characterized by different RMS roughness, thickness, surface energy  
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14 and wettability. Differently, the proliferation rate analysis, for both normal and tumor cells, did  
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16 not show significant differences between the four types of pristine substrates (Supporting  
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18 Information), therefore indicating that the morphology of the PEDOT:PSS surfaces does not  
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20 influence the growing capability of these cells.  
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24 It is interesting to note that even if the hydrophilicity of the PH1000 pristine form is  
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26 significantly lower compared to CPP105D-based films, also in this case, the proliferation rate of  
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28 both cells population is not affected.  
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31 From the data acquired we can also observe that the application of an external bias able to  
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33 change the redox state of the polymer does not affect surface morphology, energy and  
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35 hydrophilicity with respect to the pristine one; while the sheet resistance is strongly increased  
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37 only by the application of an oxidizing potential in all substrates. Nevertheless, since the  
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39 proliferation rates are not modified by any of the oxidized substrates except for the hDF growing  
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41 on PH1000, we can exclude a causal relationship between sheet resistance and proliferation  
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43 capability on biased PEDOT:PSS. On the basis of these evidences, it seems that the  
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45 electrochemical status is an independent perturbing key factor able to induce a detectable  
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47 modification of biological systems like the one used. The dependency of cell response to the  
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49 applied bias used to reduce the PEDOT films was preliminarily assessed by growing T98G cells  
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51 on ED4 substrates that had been biased at -0.4 V. While the difference of normalized cell number  
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at 72 h between not-biased and films reduced at -0.9 V is  $1.3 \pm 0.4$ , in the case of films reduced at -0.4 V it drops to  $0.3 \pm 0.4$ . This result implies that a relatively high potential is necessary to actually influence T98G cell behavior, meaning that cell replication is redox intensity-dependent.



**Figure 6.** Effect, after 72 hours from seeding, on the proliferation rate of the PEDOT:PSS substrates altered by an oxidation (+0.8 V) or reduction (-0.9 V) process. a) Proliferation rate of hDF cells plated on not biased PEDOT:PSS CPP105D (n=4), ED4 (n=4), ED8 (n=6), PH1000 (n=4) and polystyrene wells substrate (control, n=6) measured as the mean ( $\pm$  S.E.M.) number of adherent cells counted from different focal fields at 100 $\times$  of magnification at 24/48/72 hours and normalized to the values obtained from each substrate at 24 h. b) Proliferation rate of T98G cells plated on not biased PEDOT:PSS CPP105D (n=7), ED4 (n=6), ED8 (n=6), PH1000 (n=6) and polystyrene wells substrate (control, n=5), measured as described in (a). The P values are calculated by a Student T-test.

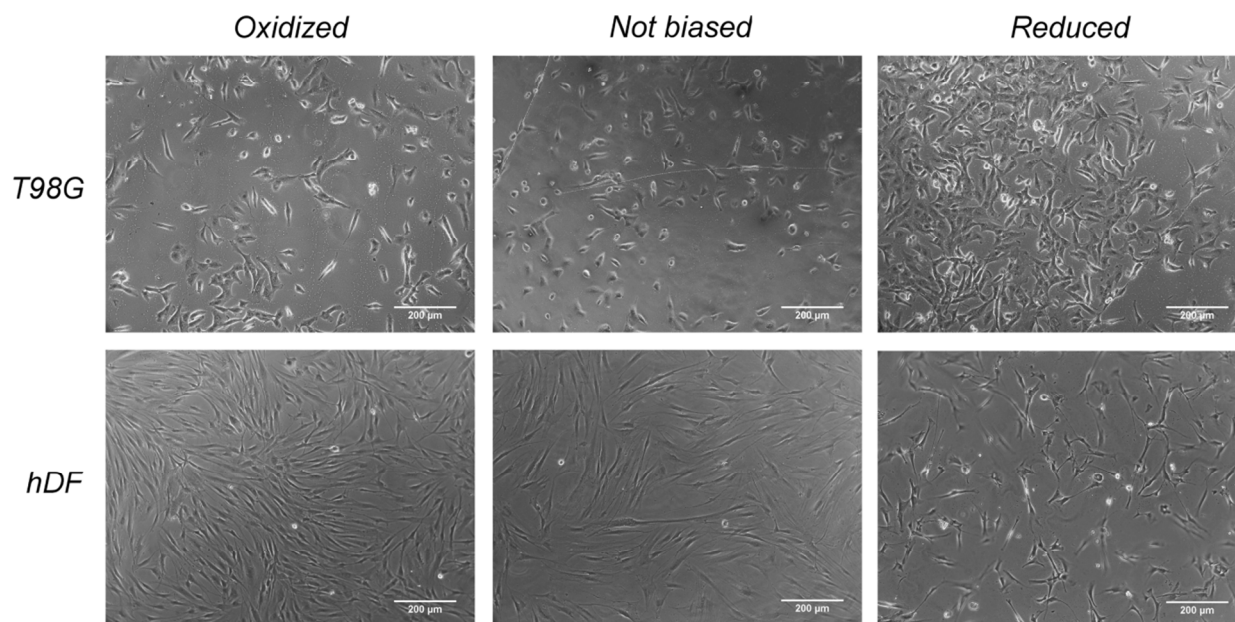
Furthermore, the interesting finding that hDF proliferation rate increases only when seeded on oxidized PH1000, while the reduced form of any PEDOT:PSS type tested is able to induce an

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3 increase only in T98G cells, suggests a different mechanism by which biased substrates might  
4 influence the replication processes of different type of cells.  
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8 The study of the electrochemical stability indicates that only the oxidized form of PH1000  
9 spontaneously undergoes reduction when exposed to the cell growth medium (DMEM) (Figure  
10 3). This state-change might be explained as an exchange of charge (i.e. ions and charged  
11 molecules) with the cell culture medium, also supported by the fact that the charge carriers  
12 acquire larger mobility in the oxidized form of PH1000, and responsible for the creation of a  
13 gradient in the ionic concentration which influences both the local chemical composition of the  
14 environment and the charge distribution of the extracellular matrix. These effects have been  
15 proven to influence fibronectin conformation<sup>10,11,34</sup> affecting adhesion and growth<sup>35,36</sup> of very  
16 sensitive cells such as primary hDF.  
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29 Furthermore, cellular adhesion phase is mediated by transmembrane proteins named integrins  
30 interacting with the extracellular matrix and the intracellular environment. It is known that  
31 whether a cell will undergo a pro-survival or a pro-apoptotic pathway depends on the ligation  
32 status that the surface integrins express. Cooperative signaling between growth factor receptors  
33 and integrins also differentially activates the proto-oncogene serine/threonine-protein kinase Raf  
34 (by a phosphorylation of specific sites) leading to distinct mechanisms of cell survival. Since it  
35 has been shown that integrin expression can vary considerably between normal and tumor  
36 tissues,<sup>37</sup> our results might be interpreted as a different effect of the reduced substrate on the  
37 integrins ligation status.  
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50 However, further studies are necessary to gain a better understanding of the processes of  
51 cellular signaling with conducting polymers, which are beyond the scope of this paper.  
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**Figure 7.** A representative example of T98G and hDF phase contrast microscopy images at 100 $\times$  of magnification, hDF and T98G proliferation on +0.8 V (oxidized), not biased and -0.9 V (reduced) PH1000 after 72 h from cell seeding. Scale bar 200  $\mu$ m.

#### 4. Conclusion

A thorough characterization of the major physical parameters of PEDOT:PSS films prepared with four different formulations and methods (Table 2), namely surface roughness, sheet resistance, wettability and surface energy, was carried out, together with an analysis of their electrochemical properties performed by the acquisition of cyclic voltammeteries and visible-NIR absorption spectra. These films were deposited by spin coating using two different commercial formulations, Clevios CPP105D and Clevios PH1000, and by electrochemical polymerization, a fabrication method that allowed us to obtain all-PEDOT:PSS films with physical and electrochemical properties that were quite different from the spin coated samples. The effect of modifications in the redox state of PEDOT:PSS on its physical and electrochemical properties was assessed for different aqueous media (distilled water, PBS and DMEM).

Electrochemical analyses showed that the oxidized (i.e. positively biased) form of PEDOT:PSS is more stable in time than the reduced (i.e. negatively biased) form when exposed to an aqueous medium, and a redox potential between -0.2 V and +0.6 V was found to give a very reproducible response in cyclic voltammetries. However, higher potentials (-0.9 V and +0.8 V) were used in order to maximize the differences between reduced, not biased and oxidized forms of PEDOT:PSS both in terms of physical properties and influence on cell growth.

Surface morphology of PEDOT:PSS films was found to be affected by the deposition technique used. Surface roughness ranged from 6 nm for CPP105D to 33 nm for ED8, increasing with an increasing number of electropolymerization cycles. On the other hand, the redox state of the polymer did not affect its surface morphology.

Static water contact angle measurements showed that wettability and surface energy significantly decreased when PEDOT:PSS interacted with PBS, while no significant change was induced by the application of an external bias.

Sheet resistance was strongly affected by the application of an oxidizing potential, which induced an increase in the electrical resistance of about two orders of magnitude in all the samples observed. The exposure to cell culture medium for 48 h induced an increase of the sheet resistance by a factor of two in all the types of PEDOT:PSS.

Cell growth experiments were carried out to assess the effectiveness of the here studied four different types of PEDOT:PSS films as substrates for controlled cell growth applications. We used two different cell lines, primary human dermal fibroblasts (hDF) and glioblastoma multiforme cells (T98G). The cells were seeded on reduced, not biased and oxidized PEDOT:PSS thin films, and the effect of the redox status on cell growth was assessed after 72 h.

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3 The result was found to be strongly cell-dependent, suggesting that each cell type has its own  
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5 peculiar response to the same environment:  
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8 hDF proliferation rate was significantly enhanced specifically by oxidized PH1000, the only  
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10 substrate that we have observed to strongly exchange charge with the cell culture medium.  
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12 T98G cells proliferation rate was significantly enhanced on reduced samples, irrespective of  
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14 the material used as substrate.  
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17 These results indicate that the cells proliferation rate has a clear dependence on the  
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19 electrochemical state of its substrate, while it is not affected by the other parameters as surface  
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21 roughness, surface conductivity and surface energy, that are very similar between not biased and  
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23 reduced/oxidized PEDOT:PSS. As a confirmation, no significant difference in the cell growth  
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25 rate is observed between the not biased forms of the four types of PEDOT:PSS films here used  
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27 as substrates, even if they show different surface parameters.  
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31 The results presented in this work allow to gain a better understanding on the major physical  
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33 and electrochemical properties of PEDOT:PSS, and how they are influenced by the interaction  
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35 with aqueous environments and by the application of a redox voltage. Furthermore, we  
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37 highlighted the importance of a precise control in the substrate deposition and oxidation  
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39 parameters and how the substrate properties are capable to drive cells activity, like growth,  
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41 taking into account the peculiar features of each type of cell. A systematic study of conjugated  
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43 polymers and their interactions with living cells is crucial to bring order into the current  
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45 scattering of the results presented in recent literature (Table 1), so as to allow the development of  
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47 new applications that can take advantage of the favorable properties of these materials.  
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ASSOCIATED CONTENT

**Supporting Information.**

Cyclic voltammetries at different scan rates of PEDOT:PSS; absorption spectra of ED4 taken at different times after polarization; trends over time of the intensity of the absorption peaks of reduced and oxidized PEDOT:PSS in different electrolytes; percentages of residual PEDOT in the reduced and oxidized forms of PEDOT:PSS after one-hour dipping in different electrolytes; absorption spectra of reduced, not biased and oxidized PEDOT:PSS after 48 h of immersion in cell culture medium; sheet resistance of PEDOT:PSS oxidized using different redox biases and its trend over time for up to 48 h in cell culture medium; comparison between growth rate of hDF and T98G cells on control Petri dish and not biased PEDOT:PSS over time. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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**Author Contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

**Notes**

The authors declare no competing financial interest.

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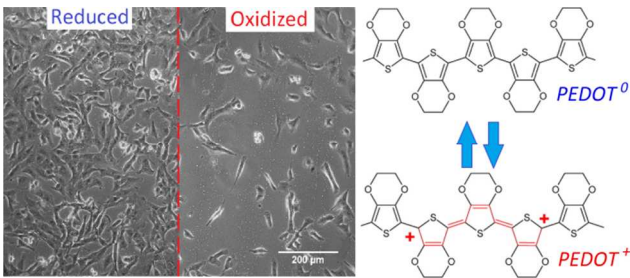
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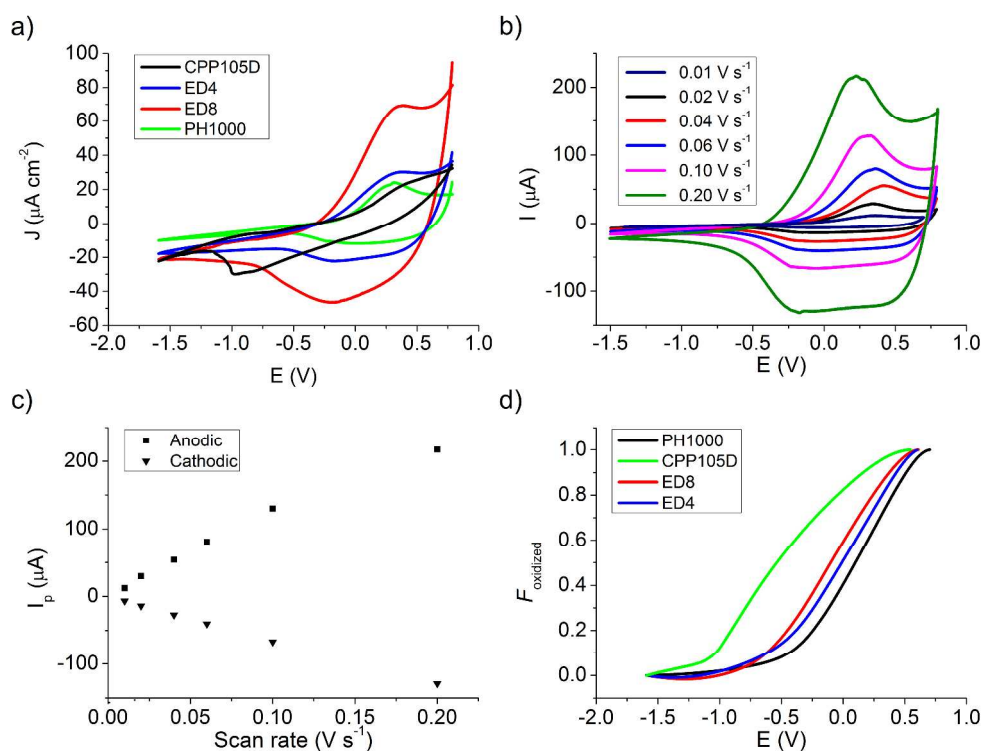
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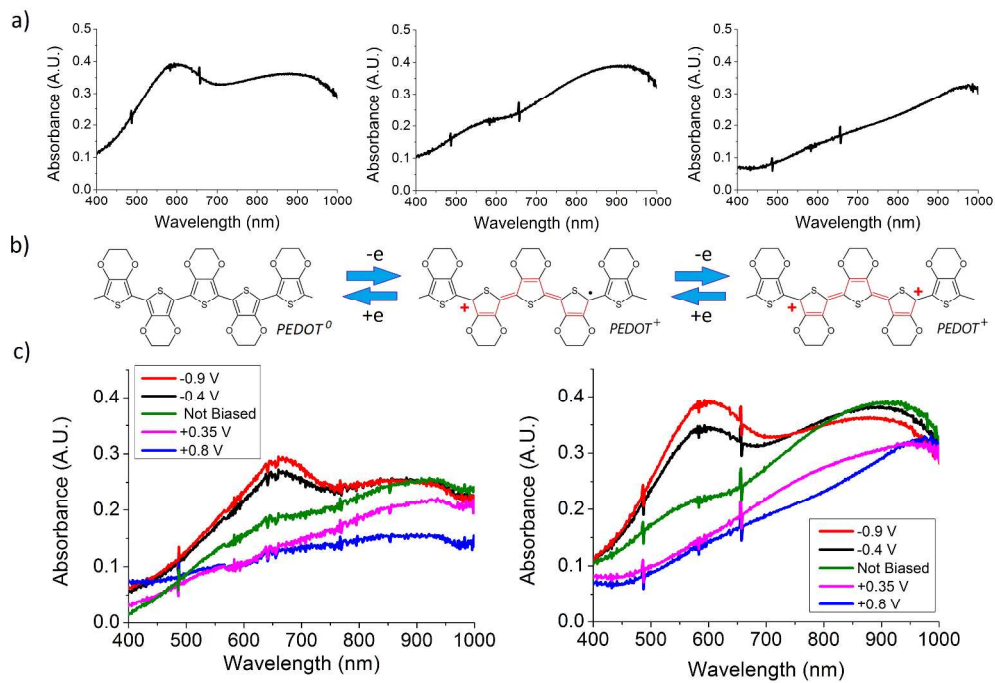
Table of Contents Graphic



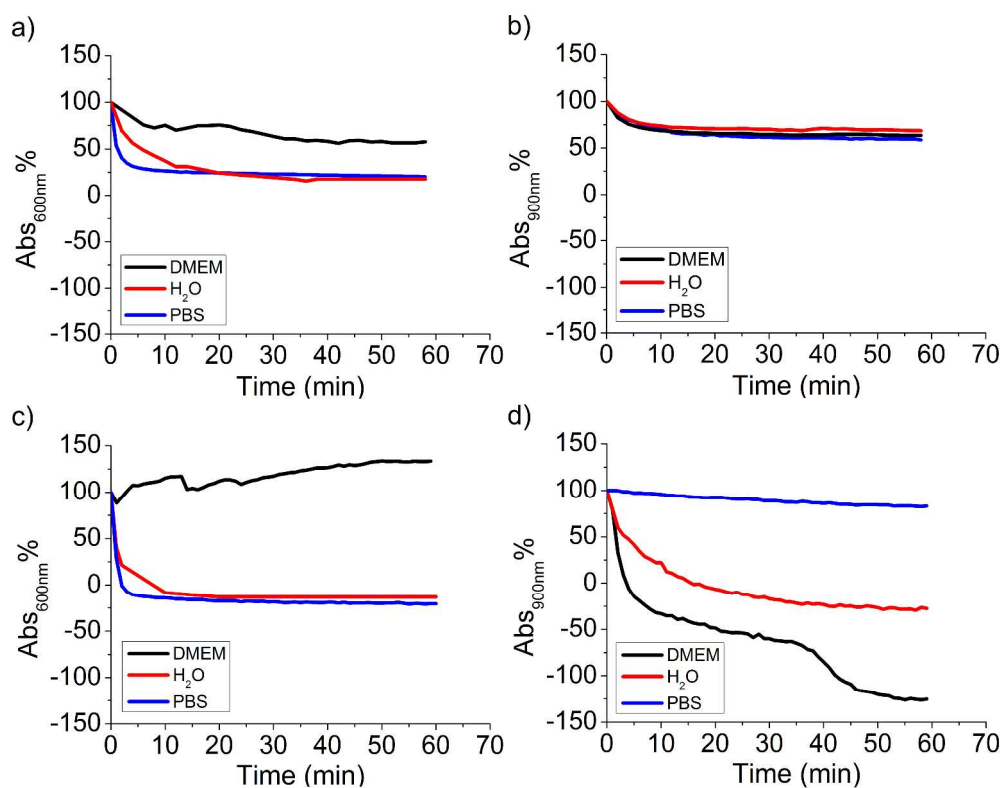


**Figure 1** a) CVs recorded for different PEDOT:PSS substrates (scan rate 20 mV s<sup>-1</sup>). b) Cyclic voltammograms of PH1000 taken at different scan rates. c) Peak current vs. scan rate. d) Fraction of oxidized PEDOT:PSS ( $F_{\text{oxidized}}$ ) for the different substrates as a function of the applied voltage. All the graphs refer to measurements carried out in PBS



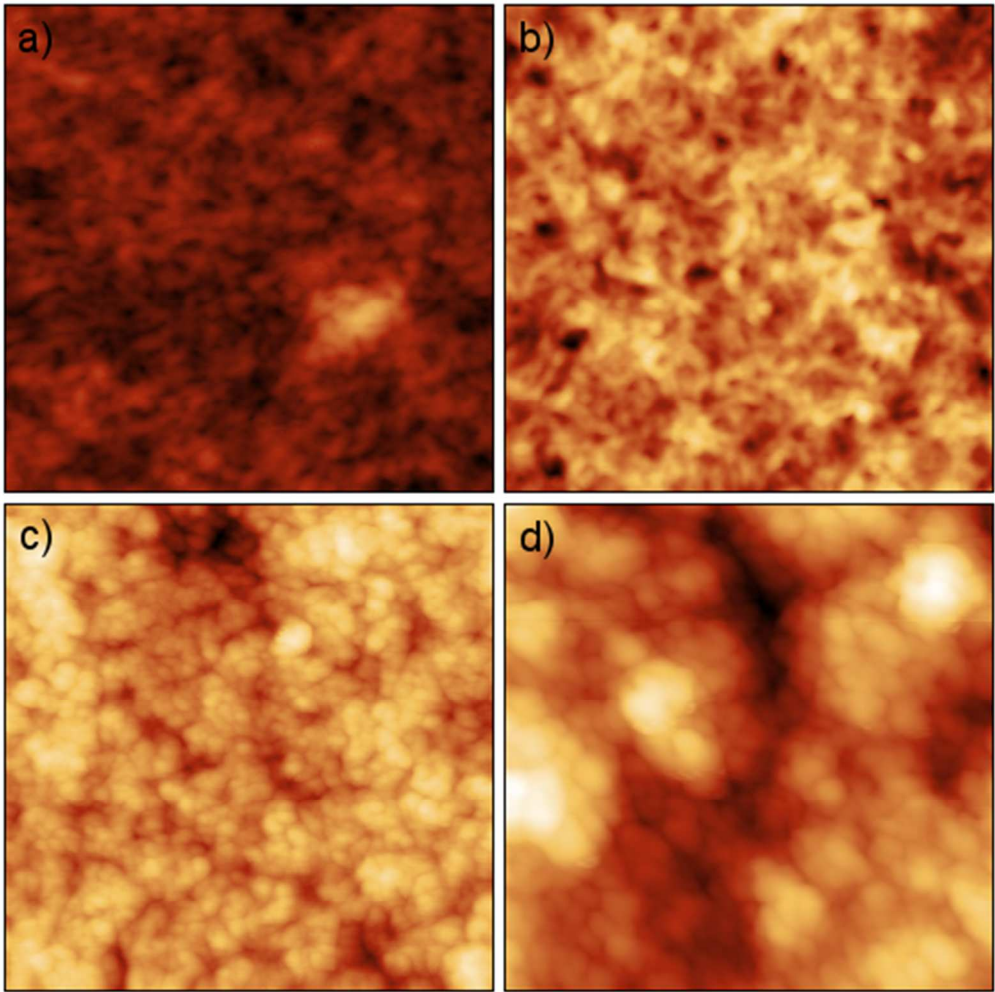


**Figure 2** a) Visible and NIR spectra of ED8 in its reduced (-0.9 V, left), not-biased (middle) and oxidized (+0.8 V, right) forms. b) Schematics of the redox process in PEDOT from its reduced (left) to its fully oxidized (right) state. c) Visible and near IR spectra of PH1000 (left) and ED8 (right) in their native state and biased at -0.9 V, -0.4 V, +0.35 V and +0.8 V.

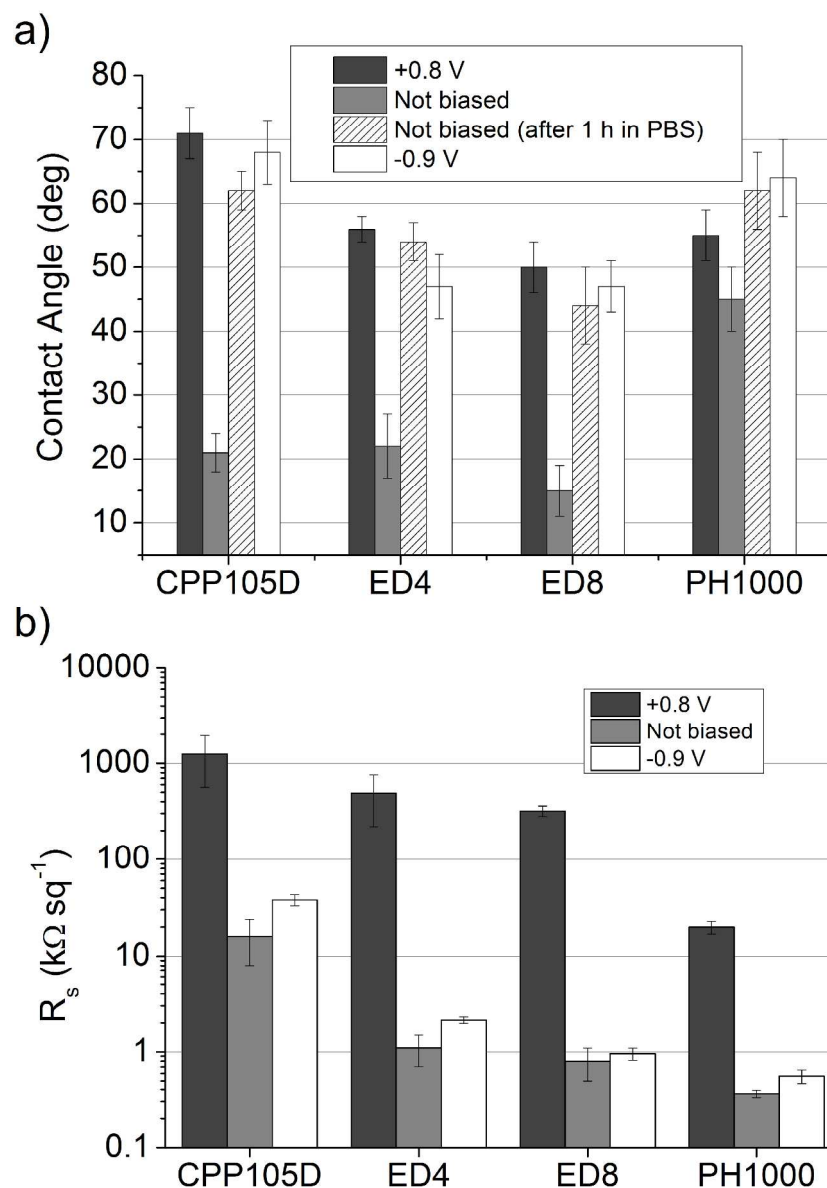


**Figure 3** Trends of  $Abs_{600nm}\%$  and  $Abs_{900nm}\%$  for (a) reduced ED4, (b) oxidized ED4, (c) reduced PH1000, and (d) oxidized PH1000. The samples were biased at -0.9 and +0.8 V, and then immersed in DMEM,  $H_2O$  and PBS. The trends for CPP105D and ED8 are available in the Supporting Information.

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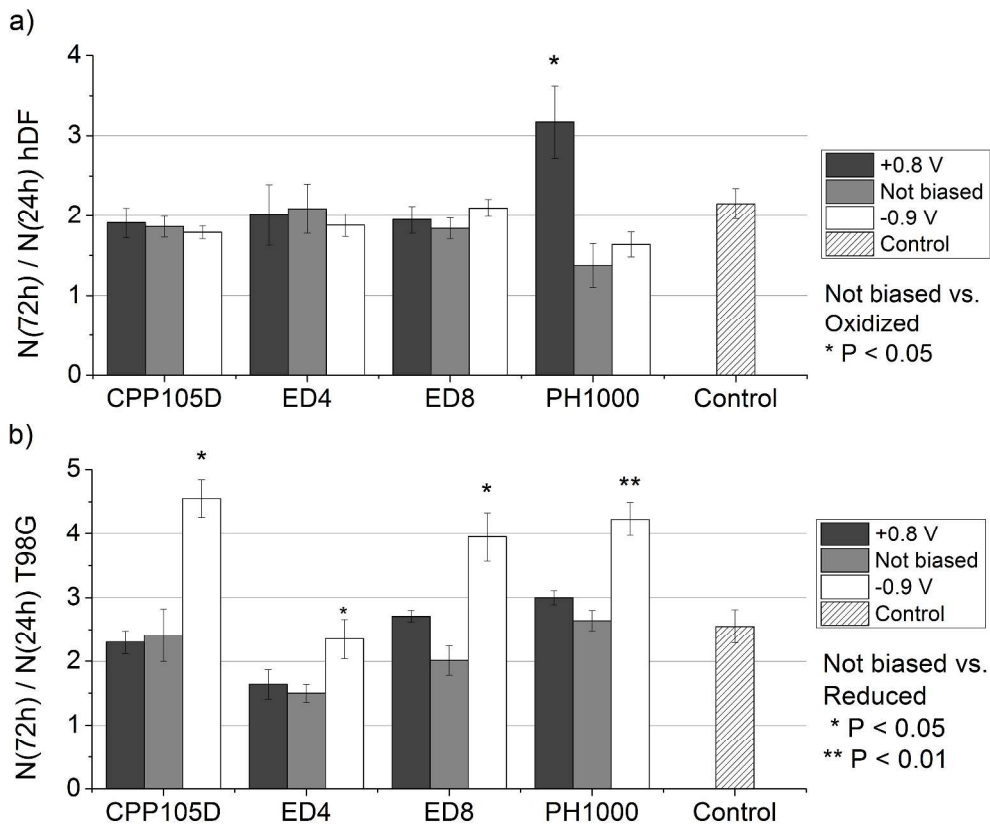


**Figure 4** AFM topography images of the PEDOT:PSS samples under study: a) PH1000, b) CPP105D, c) ED4, d) ED8. Scan size: 1 $\mu$ m x 1 $\mu$ m, z scale: 35 nm for (a), (b) and (c), 90 nm for (d). 44x44mm (300 x 300 DPI)



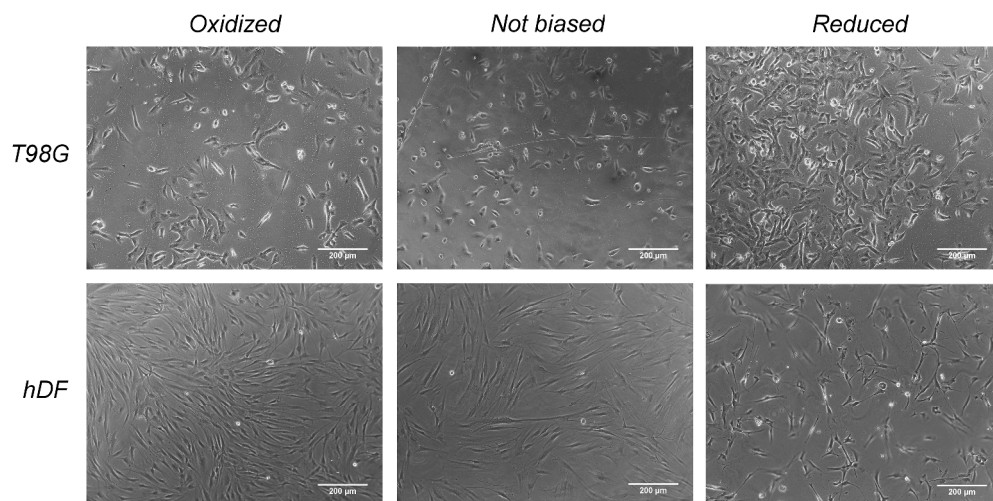
**Figure 5** a) Water static contact angle measured on the PEDOT:PSS samples under study in their oxidized, not biased and reduced form. The not biased samples were measured as deposited and after being immersed in PBS for 1 h. b) Sheet resistance measured on all the four different sets of PEDOT:PSS samples here investigated, in their oxidized, not biased and reduced form.

202x282mm (300 x 300 DPI)



**Figure 6** Effect, after 72 hours from seeding, on the proliferation rate of the PEDOT:PSS substrates altered by an oxidation (+0.8 V) or reduction (-0.9 V) process. a) Proliferation rate of hDF cells plated on not biased PEDOT:PSS CPP105D (n=4), ED4 (n=4), ED8 (n=6), PH1000 (n=4) and polystyrene wells substrate (control, n=6) measured as the mean ( $\pm$  S.E.M.) number of adherent cells counted from different focal fields at 100x of magnification at 24/48/72 hours and normalized to the values obtained from each substrate at 24 h. b) Proliferation rate of T98G cells plated on not biased PEDOT:PSS CPP105D (n=7), ED4 (n=6), ED8 (n=6), PH1000 (n=6) and polystyrene wells substrate (control, n=5), measured as described in (a). The P values are calculated by a Student T-test.

449x377mm (300 x 300 DPI)



**Figure 7** A representative example of T98G and hDF phase contrast microscopy images at 100× of magnification, hDF and T98G proliferation on +0.8 V (oxidized), not biased and -0.9 V (reduced) PH1000 after 72 h from cell seeding. Scale bar 200 μm.  
458x229mm (300 x 300 DPI)