



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

ARCHIVIO ISTITUZIONALE
DELLA RICERCA

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

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

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Physical and Electrochemical Properties of PEDOT:PSS as a Tool for Controlling Cell Growth / Marzocchi, M.; Gualandi, I.; Calienni, M.; Zironi, I.; Scavetta, E.; Castellani, G.; Fraboni, B.. - In: ACS APPLIED MATERIALS & INTERFACES. - ISSN 1944-8244. - STAMPA. - 7:32(2015), pp. 17993-18003. [10.1021/acsami.5b04768]

Availability:

This version is available at: <https://hdl.handle.net/11585/518091> since: 2015-11-06

Published:

DOI: <http://doi.org/10.1021/acsami.5b04768>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).
When citing, please refer to the published version.

(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

Marco Marzocchi, Isacco Gualandi, Maria Calienni, Isabella Zironi, Erika Scavetta, Gastone Castellani, and Beatrice Fraboni, *Physical and Electrochemical Properties of PEDOT: PSS as a Tool for Controlling Cell Growth*. ACS Applied Materials & Interfaces 2015, 7 (32), 17993-18003.

The final published version is available at: <https://doi.org/10.1021/acsami.5b04768>

Rights / License:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

When citing, please refer to the published version.

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

Marco Marzocchi,^{*,†} Isacco Gualandi,[†] Maria Calienni,[†] Isabella Zironi,[†] Erika Scavetta,[‡]

Gastone Castellani,[†] and Beatrice Fraboni[†]

[†] Department of Physics and Astronomy, University of Bologna, viale Berti-Pichat 6/2, 40127

Bologna, Italy

[‡] Department of Industrial Chemistry, University of Bologna, viale Risorgimento 4, 40136

Bologna, Italy

*Email: marco.marzocchi4@unibo.it

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

1
2
3 electrochemical properties of these different types of PEDOT:PSS are studied through cyclic
4
5 voltammetry and spectrophotometry to assess the effectiveness of the oxidation process and its
6
7 stability over time. Their surface physical properties and their dependence on the redox state of
8
9 PEDOT:PSS are investigated using atomic force microscopy (AFM), water contact angle
10
11 goniometry and sheet resistance measurements. Finally, human glioblastoma multiforme cells
12
13 (T98G) and primary human dermal fibroblasts (hDF) are cultured on PEDOT:PSS films with
14
15 different oxidation states, finding that the effect of the substrate on the cell growth rate is
16
17 strongly cell-dependent: T98G growth is enhanced by the reduced samples, while hDF growth is
18
19 more effective only on the oxidized substrates that show a strong chemical interaction with the
20
21 cell culture medium.
22
23
24
25
26
27

28 **1. Introduction**

29
30
31 Thanks to their favorable properties, namely chemical stability, low temperature processing,
32
33 oxide-free surface in aqueous electrolyte, ionic and electronic transport, and mechanical
34
35 compliance with living tissues, conjugated polymers (CPs), are promising materials for tissue
36
37 engineering applications and are gaining more and more interest for the development of
38
39 biosensors and bioactuators.¹⁻³ Among the many existing conducting polymers, poly(3,4-
40
41 ethylene dioxythiophene) (PEDOT)-based materials, and especially its polystyrenesulfonate-
42
43 doped (PEDOT:PSS) and p-toluenesulfonate-doped (PEDOT:TOS) forms, have become
44
45 reference materials for the interfacing of electronics and living tissues. Over the last few years,
46
47 many studies involving the effect of the oxidation state of CPs on cell adhesion, density and
48
49 replication have been carried out.⁴⁻¹⁰ In 1994, Wong *et al.* demonstrated that it is possible to
50
51 control living cell growth through a change in the oxidation state of polypyrrole used as substrate
52
53 for cell culture.⁴ More recently, it has been shown that this effect involves a change in density
54
55
56
57
58
59
60

1
2
3 and conformation of adhesion proteins, which is strictly related to the oxidation state of the
4
5 conducting polymer used as substrate.^{10,11} It is known that cell-substrate interaction depends on
6
7 the surface properties of the substrate itself: wettability,⁵ surface roughness¹²⁻¹⁴ and electrical
8
9 conductivity^{15,16} are key parameters in regulating this interaction, but their specific role in the
10
11 adhesion process, together with the effect of electrochemical kinetics and protein-substrate
12
13 interactions, are still not completely understood. For this reason, a systematic characterization of
14
15 the surface properties of conjugated polymers, and more specifically on the dependency of these
16
17 properties with respect to the oxidation state of conjugated polymers, would be of critical
18
19 importance in order to disentangle the role of each different parameter involved in the cell-
20
21 substrate adhesion process. However, a systematic approach to this problem is still lacking in
22
23 literature. As can be seen from Table 1, where some of the main papers published in the last
24
25 twenty years about the effect of the redox state of conjugated polymers on cell adhesion and
26
27 growth are summarized, each study is carried out in different experimental conditions, in terms
28
29 of material used, oxidation process, cell line observed and cell growth conditions. Such
30
31 variability in sample preparation and biological conditions brought about the observed very
32
33 scattered results, all of them confirming that it is possible to control cell adhesion and growth,
34
35 but hindering the possibility to draw general conclusions. Moreover, although the
36
37 electrochemical properties of conductive polymers are exploited in several applications, such as
38
39 electrochemical transistors,¹⁷ actuators,¹⁸ electrochromic devices,¹⁹ smart membranes and drug
40
41 delivery devices,²⁰ the physical and chemical processes involved in the doping/dedoping
42
43 reactions have not yet been clarified. The aim of the present work is to assess the effects induced
44
45 by a change in the redox state of PEDOT:PSS on the main physical parameters which are related
46
47 to the cell adhesion process, which we identified as surface morphology, electrical conductivity,
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 wettability and oxidation state, and to show that a precise control of these conditions is possible
4 and necessary to achieve reproducible results. We therefore carried out cell growth experiments
5
6 on four different types of PEDOT:PSS using two different cell lines, primary human dermal
7
8 fibroblasts (hDF) and human glioblastoma multiforme cells (T98G). These types of cells have
9
10 been chosen in order to assess the effects of the substrates parameters on adherent, high-
11
12 replicating and tissue-forming cells with very similar genotypic and phenotypic properties but
13
14 normal (hDF) and tumor-like (T98G).
15
16
17
18

19 20 **2. Experimental Section**

21
22 *Materials:* Clevios CPP105D and Clevios PH1000 were purchased from Heraeus. Ethylene
23
24 glycol, dodecyl benzene sulfonic acid (DBSA), 3-glycidoxypropyl trimethoxysilane (GOPS),
25
26 EDOT monomer, PSS and phosphate buffer saline solution (PBS) were purchased from Sigma-
27
28 Aldrich.
29
30

31
32 *Fabrication of the PEDOT:PSS substrates:* Two different formulations of PEDOT:PSS were
33
34 used, Clevios CPP105D and Clevios PH1000. The former is a low conductive formulation,
35
36 which is optimized to be deposited on hydrophobic substrates, while the latter is optimized to be
37
38 highly conductive and was mixed with ethylene glycol (20 v/v %) and DBSA (0.05 v/v %) in
39
40 order to improve its electrical conductivity and the ease of the spin coating deposition. A cross-
41
42 linking agent, GOPS 1 v/v %, was added to both CPP105D and PH1000 in order to improve their
43
44 resistance to delamination when exposed to water. These two suspensions were treated in
45
46 ultrasonic bath for 10 minutes and filtered using 1.2 μm cellulose acetate filters (Sartorius), then
47
48 spin coated over clean glass slides at 1000 rpm for 10 s. The samples were subsequently dried at
49
50 140 °C for 30 minutes to remove water and other solvents.
51
52
53
54
55
56
57
58
59
60

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

^{a)} Fn: fibronectin; PEG: polyethylene glycol; ^{b)} FBS: fetal bovine serum; ^{c)} NaDBS: Sodium dodecylbenzenesulfonate.

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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 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

1
2
3 rinsed in distilled water and dried at room temperature. It must be noted that the use of a
4
5 reference electrode during the biasing of the samples was proven to be essential to achieve a
6
7 reproducible oxidation state of PEDOT:PSS, since without this reference, the actual chemical
8
9 potential of the polymer films is basically random. Different biases were used to study the
10
11 voltage dependence of the physical and chemical properties of this polymer, and their different
12
13 effect on cell replication. In the present work, only data acquired from the samples treated with
14
15 the highest potentials we tested, $V = +0.8$ V (oxidized) and $V = -0.9$ V (reduced), are reported,
16
17 since they show the most significant effect.
18
19
20
21

22 *Electrochemical characterization:* All the electrochemical experiments were carried out in a
23
24 three electrode cell equipped with a SCE as reference electrode and a Pt wire as counter
25
26 electrode. The responses were recorded using PGSTAT20 (Ecochemie) connected to a personal
27
28 computer. The electrochemical characterizations were performed by cyclic voltammetry between
29
30 -1.6 V and +0.8 V employing a scan rate of 20 mV s^{-1} . The samples characterized by CV were
31
32 used only for such experiments, and not for any further characterization. The stability of the
33
34 electrochemically-generated redox states were evaluated by spectroelectrochemical experiments
35
36 using a HewlettPackard 8453 diode array spectrophotometer. The samples were biased for 1h by
37
38 applying a continuous voltage in PBS, and subsequently Vis-NIR (300-1000 nm) spectra were
39
40 collected for 60 min every 1 min after the end of the polarization.
41
42
43
44
45

46 *Atomic force microscopy:* Imaging of the surface of the PEDOT:PSS films was carried out
47
48 using a Park NX10 atomic force microscope (AFM) operating in non-contact mode in air in
49
50 ambient conditions. Root mean square roughness (R_q) and thickness (t) of the polymer films
51
52 were evaluated through the acquisition of topographical images with scan size $5 \mu\text{m} \times 5 \mu\text{m}$. In
53
54
55
56
57
58
59
60

1
2
3 order to assess the homogeneity of the electrodeposition process, scans were performed at three
4
5 different positions on each film for several samples.
6
7

8 *Wettability measurements:* Hydrophilicity and surface energy of the PEDOT:PSS films were
9
10 determined by water contact angle measurements (surface tension of 72.8 mN m^{-1}), which were
11
12 performed with a custom setup using the sessile drop technique. The average contact angle value
13
14 was obtained by measuring the contact angles at two different positions on two different
15
16 samples, and the error on the average was evaluated as the standard deviation of this set of
17
18 measurements for each material. The contact angles were converted into surface energy values
19
20 employing the relation of E. Chibowski *et al.*²¹
21
22
23

24 *Surface resistivity measurements:* The sheet resistance (R_s) of the polymer films was measured
25
26 using a 4-probe custom setup, where four aligned and evenly spaced (spacing 2.7 mm)
27
28 conductive tips were connected to a Keithley 2400 SourceMeter, which was used as a current
29
30 generator for the two external tips and as a voltage meter for the two inner tips. Sheet resistance
31
32 was measured at three different positions for each sample on at least three samples. An ideal
33
34 geometrical correction factor of $\pi/\ln 2$ was applied to all measurements.²² The reported error on
35
36 the average was evaluated as the standard deviation of the set of measurements carried out for
37
38 each material.
39
40
41
42

43 *Cell culture:* Glioblastoma multiforme cells (T98G), derived from a human tumor, were
44
45 maintained in Roswell Park Memorial Institute (RPMI) medium, supplemented with 10% fetal
46
47 bovine serum, 1% L-glutamine, 10% sodium pyruvate and antibiotics (1% penicillin and 1%
48
49 streptomycin). Primary dermal fibroblast (hDF), gently provided by Prof. S. Salvioli (DIMES,
50
51 University of Bologna), were obtained from two donors (age 24 and 23 years) by skin biopsies
52
53 according to standard culture method. All the donors gave their informed consent before biopsy
54
55
56
57
58
59
60

1
2
3 was performed. hDF were maintained in Dulbecco's modified Eagle's medium (DMEM),
4 supplemented with 10% fetal bovine serum, 1% L-glutamine and antibiotics (1% penicillin and
5
6 1% streptomycin). hDF of 3-15 passages number were used. Both cells population were kept in
7
8 culture standard conditions in incubator at 5% CO₂ humidified atmosphere at 37°C. Before
9
10 experiment cells were detached by trypsin (0.02%) in ethylenediaminetetraacetic acid (EDTA)
11
12 and re-suspended in culture medium at room temperature (all reagents were purchased from
13
14 Sigma Chemical Italy, Milano, Italy)
15
16
17
18
19

20 *Proliferation curves:* the cell culture 24-wells plates (CELLSTAR, Greiner bio-one) were
21 prepared as follow: PEDOT:PSS substrates in its non-biased, oxidized and reduced form were
22
23 cut using a diamond tip and three replication for each form were placed in nine different wells of
24
25 the plate; the plate were sterilized under UV per 20 min. Before cells seeding the PEDOT:PSS
26
27 was washed out with sterile culture medium in order to buffer the pH altered by the substrates.
28
29
30
31

32 T98G and hDF cells were counted by an hemocytometer and seeded in a density of $0.010 \times$
33
34 10^6 and 0.015×10^6 with 500 μ l of supplemented RPMI and DMEM, respectively, in each well
35
36 prepared as described and in three wells without PEDOT:PSS, i.e. on a sterile hydrophilic
37
38 polystyrene tissue culture surface (control) physically treated for improving cell adhesion. Cells
39
40 were allowed to adhere and spread for 24 h prior the observation.
41
42
43

44 We studied cell proliferation by growing T98G and hDF in a CO₂ incubation system integrated
45
46 within a motorized stage allowed to perform time-lapse imaging acquisition even for tens of
47
48 hours. Images were acquired in phase-contrast at 100 \times of magnification for a time interval up to
49
50 168 h (seven days) by the inverse automatized optical microscopy Nikon Eclipse-Ti (Nikon,
51
52 Italy).
53
54
55
56
57
58
59
60

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 ± 0.3 V and ± 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 ⁻³) ^{a)}
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

^{a)} 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⁻¹ (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.²³ Therefore, the charge

1
2
3
4
5
6 flowing during the CV scans corresponds to the electrons that are needed to change the redox
7
8 state of the whole PEDOT:PSS volume. Consequently, the degree of electrochemical doping can
9
10 be calculated for the different materials per each potential. From Figure 1d, which reports the
11
12 fraction of oxidized PEDOT sites over the number of total active sites (i.e. where electrons are
13
14 exchanged), it is possible to see that the active sites that take part in the redox processes are
15
16 almost completely reduced ($F_{oxidized} \approx 0$) at -0.9 V. On the other hand, the anodic limit for
17
18 polarization is found at +0.8 V, since it was observed that higher potential values lead to a strong
19
20 overoxidation of the conductive polymer.
21
22
23

24
25 If we assume that only one charge interacts with each active site of the conductive polymer, it
26
27 is also possible to calculate the concentration of the sites that change their redox state by
28
29 measuring the charge that flows during the cathodic scan of a cyclic voltammetry. This
30
31 concentration, that can be directly correlated to the number of charge carriers, resulted equal to
32
33 0.55, 0.54, 0.21, 0.16 mol dm⁻³ for ED4, ED8, CPP105D and PH1000, respectively (Table 2).
34
35 The larger values of electrochemically polymerized PEDOT:PSS can be ascribed to its higher
36
37 PEDOT/PSS ratio.²⁴
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

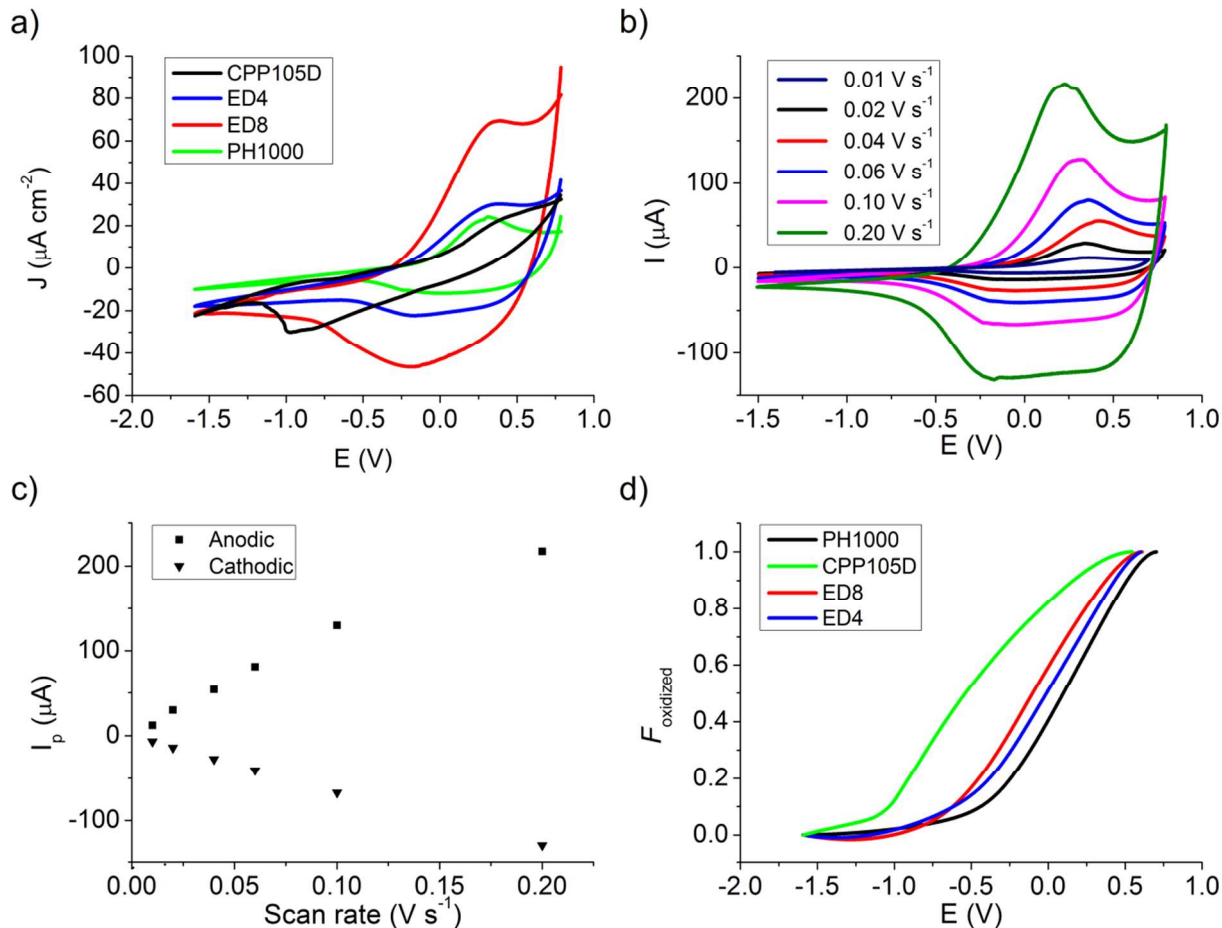


Figure 1. a) CVs recorded for different PEDOT:PSS substrates (scan rate 20 mV s⁻¹). b) Cyclic voltammograms of PH1000 taken at different scan rates. c) Peak current vs. scan rate. d) Fraction of oxidized PEDOT:PSS (F_{oxidized}) for the different substrates as a function of the applied voltage. All the 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.²⁵ 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.²⁶ 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.²⁷

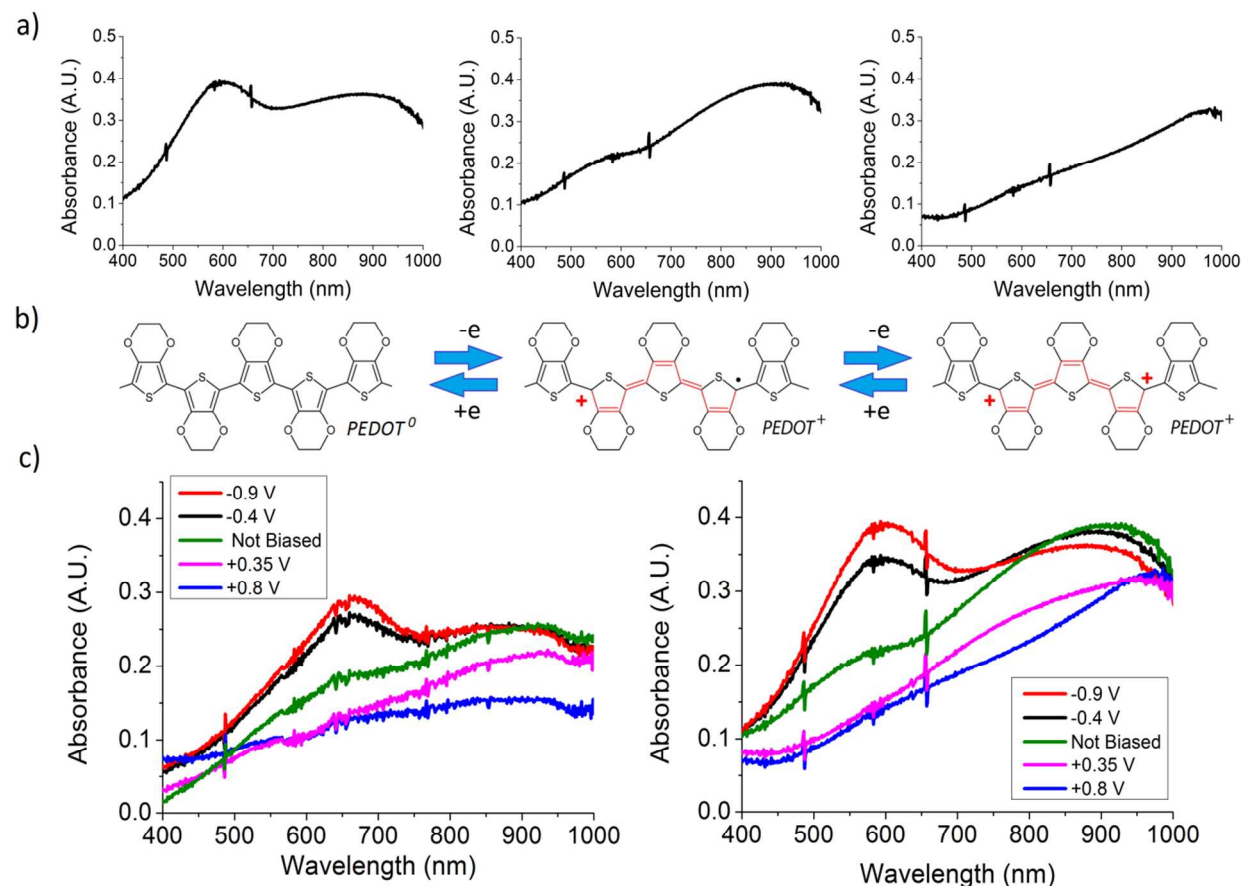


Figure 2. a) Visible and NIR spectra of ED8 in its reduced (-0.9 V, left), not-biased (middle) and oxidized (+0.8 V, right) forms. b) Schematics of the redox process in PEDOT from its reduced (left) to its fully oxidized (right) state. c) Visible and near IR spectra of PH1000 (left) and ED8 (right) in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

1
2
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,
5
6 as shown in Figure 2b. However, the band at 900 nm points out that charged polarons are still
7
8 present in PEDOT:PSS, suggesting that the reduction of this material is incomplete at -0.9 V.
9
10 This effect was explained in literature with the presence of an aqueous medium, which slowly
11
12 oxidizes PEDOT.²⁸ Finally, the spectrum recorded at -0.4 V also shows a peak at 600 nm (Figure
13
14 2c), but its intensity is slightly lower with respect to the spectrum obtained at -0.9 V, meaning
15
16 that a lower percentage of PEDOT is in its neutral form.
17
18
19
20
21

22 Having assessed the redox effects induced on PEDOT:PSS thin films, we prepared
23
24 PEDOT:PSS substrates for our cell growth experiments by choosing three applied bias values
25
26 that generate three different sets of samples: one containing mainly neutral PEDOT, one
27
28 containing mainly polarons and one wherein PEDOT is present in its high conductive state. In
29
30 order to maximize the difference in performance of these three types of substrates, PEDOT:PSS
31
32 was thus used either in its pristine state or biased at -0.9 and +0.8 V.
33
34
35

36 The stability in time of the electrochemically generated redox state of PEDOT:PSS thin films
37
38 plays a key role in their effective use as cell growth substrates. To ensure long-term stability of
39
40 the redox state, the films were biased for 1 h and we thus monitored the modifications that
41
42 occurred after the applied bias was removed, by collecting the visible and NIR spectra of
43
44 reduced/oxidized PEDOT:PSS while immersed in three different aqueous media: distilled water
45
46 (H₂O), phosphate buffer solution (PBS) and Dulbecco's modified Eagle's medium (DMEM)
47
48 supplemented with fetal bovine serum. These media were used because of their different
49
50 composition, since PBS contains small ions only while DMEM also contains quite large organic
51
52 molecules, like amino acids, vitamins and glucose. The stability of the induced redox state was
53
54
55
56
57
58
59
60

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:

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

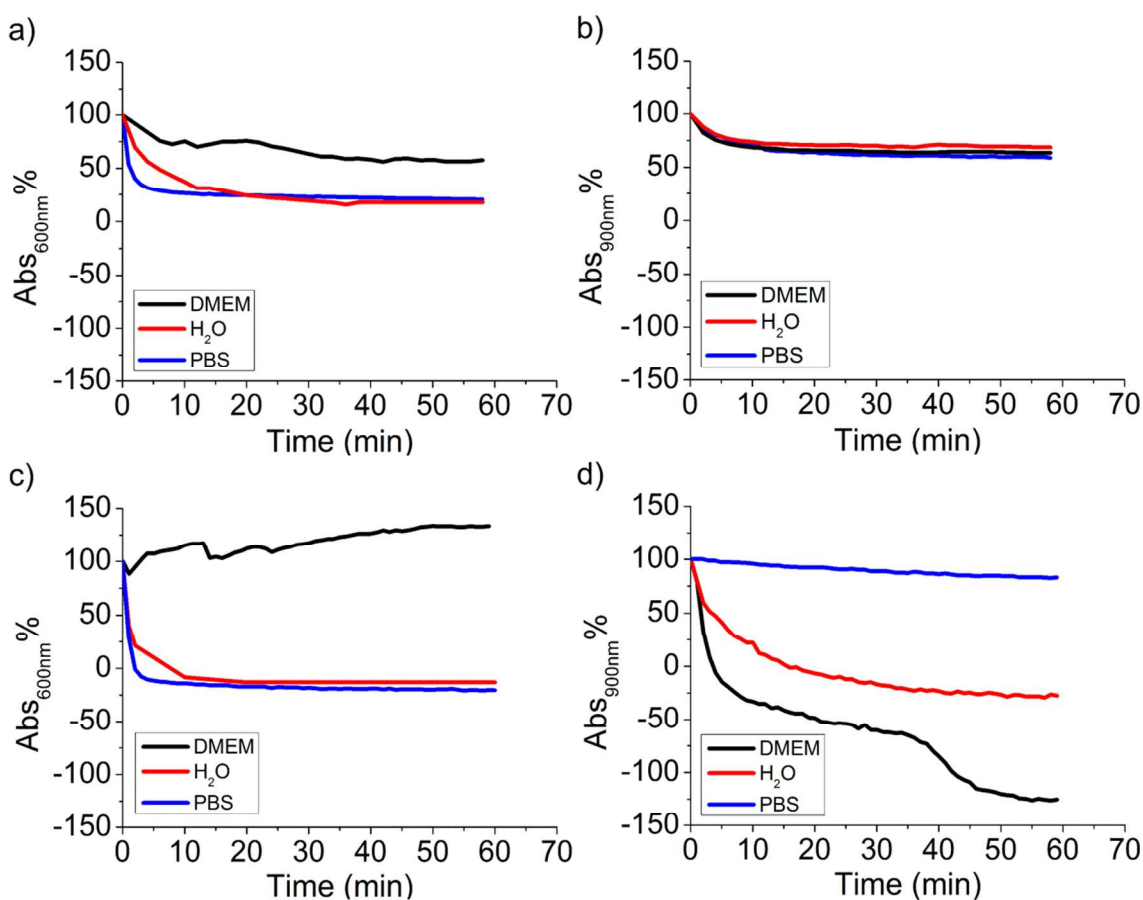


Figure 3. Trends of $Abs_{600nm}\%$ and $Abs_{900nm}\%$ for (a) reduced ED4, (b) oxidized ED4, (c) reduced PH1000, and (d) oxidized PH1000. The samples were biased at -0.9 and +0.8 V, and then immersed in DMEM, H₂O and PBS. The trends for CPP105D and ED8 are available in the Supporting Information.

1
2
3 Where $A_{600nm}(0)$ and $A_{600nm}(t)$ are, respectively, the absorbance at 600 nm of biased
4 PEDOT:PSS just after it was soaked in the medium and after being dipped for a time t , while
5
6
7
8 $A_{600nm\ native}$ is the absorbance at 600 nm of native PEDOT:PSS. Analogously, the quantity
9
10 $Abs_{900nm}\%$, also reported in Figure 3, refers to the absorbance peak at 900 nm.

11
12 The spectrum of negative-biased substrates displays a decrease of the absorbance peak at 600
13 nm that is due to the transition $\pi \rightarrow \pi^*$ of the neutral form of PEDOT generated by the
14 polarization at -0.9 V. Therefore, the amount of neutral PEDOT that is still present in the sample
15 at the time t is proportional to $Abs_{600nm}\%$.

16
17 When PEDOT:PSS is biased at +0.8 V, an increase in the peak at about 900 nm is observed
18 after the applied bias is removed, due the formation of polarons or bipolarons (Figure 2).
19 Therefore, the amount of highly conductive PEDOT:PSS that has turned in the average oxidized
20 state can be estimated by the recovery of absorbance at 900 nm, $Abs_{900nm}\%$.

21
22 Figure 3 shows that the reduced forms of PEDOT:PSS revert to their pristine state in a few
23 minutes when exposed to distilled water and PBS, while in DMEM their absorbance drops at
24 about 60% of its starting value and then stabilizes. The oxidized forms are more stable in time
25 than the reduced ones for any tested medium for substrates ED4 (Figure 3a, b), ED8 and
26 CPP105D (Supporting Information), as suggested by preliminary studies in water.^{28,29} After one
27 hour, the residual oxidation of all the samples is about 60% of the initial state, with the only
28 exception of PH1000 (Figure 3c, d). DMEM acted strongly on the redox state of oxidized
29 PH1000, leading to the reduction of PEDOT. The same measurement in DMEM was repeated by
30 monitoring the PEDOT:PSS spectrum for 48 h, in order to assess its stability in cell culture
31 medium for a time period comparable to the duration of biological experiments (Supporting
32 information). As a result, the difference in the absorption spectra of oxidized and reduced
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 samples was still observable, with a residual oxidation of about 90% of the starting value for the
4
5 oxidized samples and about 30% for the reduced ones, attesting the effectiveness of the redox
6
7 process. Moreover, the spectrum of oxidized PH1000 showed an absorbance peak between 300
8
9 nm and 400 nm after 48 h of immersion in DMEM which was not present at the beginning of the
10
11 measurement, suggesting the occurrence of a chemical modification induced by the cell culture
12
13 medium.
14
15

16
17 From these observations we can conclude that, although the generated redox states are not
18
19 completely stable in time, a sharp difference between the oxidized and reduced films is still
20
21 present after the removal of the redox voltage for all 4 types of PEDOT:PSS samples here
22
23 investigated, meaning that the application of a continuous bias during cell growth is not
24
25 necessary to grant an effective difference in the PEDOT:PSS substrate redox state.
26
27

28 29 **3.2 Surface roughness and wettability**

30
31 AFM imaging was used to evaluate the surface roughness and the thickness of thin films of
32
33 different types of PEDOT:PSS. Topography maps of the different samples are shown in Figure 4,
34
35 while the corresponding roughness and thickness values are reported in Table 3, together with
36
37 surface energy of oxidized and reduced PEDOT:PSS calculated numerically from water contact
38
39 angle measurements taken before and after the electrochemical redox process, as described
40
41 below in this paragraph.
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

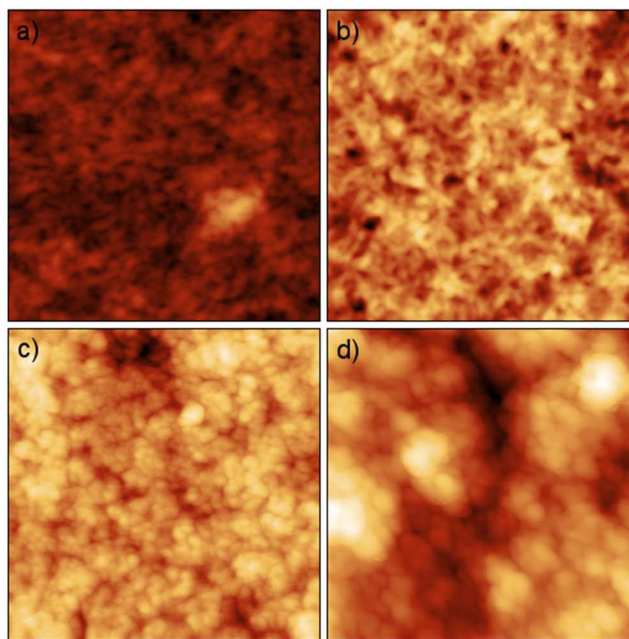


Figure 4. AFM topography images of the PEDOT:PSS samples under study: a) PH1000, b) CPP105D, c) ED4, d) ED8. Scan size: $1\mu\text{m} \times 1\mu\text{m}$, z scale: 35 nm for (a), (b) and (c), 90 nm for (d).

Redox voltage (V)	RMS Roughness (nm)	Thickness (nm)	Surface Energy (mJ m^{-2})			
			-0.9	0	0 (after 1h in PBS)	+0.8
CPP105D	6 ± 1	260 ± 70	43 ± 3	68 ± 1	47 ± 2	41 ± 3
ED4	22 ± 4	240 ± 80	56 ± 3	68 ± 2	51 ± 2	50 ± 1
ED8	33 ± 2	480 ± 120	56 ± 2	70 ± 1	57 ± 3	54 ± 2
PH1000	13 ± 3	440 ± 30	45 ± 4	57 ± 3	47 ± 3	51 ± 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 $20\mu\text{m} \times 20\mu\text{m}$. 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

1
2
3 PEDOT-rich globular structures of increasing size, as can be seen from Figure 4. The thickness
4 of the samples is basically not affected by the first four cycles of polymerization, while the
5 following four cycles increase the thickness of PEDOT:PSS by about 100% of its starting value.
6
7 This effect can be explained by assuming that polymerization starts inside the volume of
8 CPP105D, used as conductive electrode, during the first cycles of this process, and then the
9 electropolymerized PEDOT:PSS starts growing on the surface of the electrode only after a
10 certain number of cycles, increasing the overall film thickness. Finally, we preliminarily assessed
11 the effect induced by the redox process on the surface morphology of the PEDOT:PSS thin films
12 here investigated, finding no significant variation from the roughness of pristine substrates, even
13 for the highest biases used, -0.9 V and +0.8 V (data not shown).
14
15
16
17
18
19
20
21
22
23
24
25
26

27 Water contact angle measurements were carried out on the samples just after deposition
28 (pristine) and after 1 h of polarization in PBS, by applying the same potentials used in cell
29 growth experiments. As a control, some of the samples were immersed in PBS for 1 h with no
30 bias applied. The results are reported in Figure 5a. All the samples are hydrophilic (contact angle
31 is always below 90°). However, regarding the pristine form of PEDOT:PSS, there is a clear
32 difference between CPP105D-based films (both ED4 and ED8 are polymerized using CPP105D
33 films as working electrode) and PH1000, which shows a lower hydrophilicity. The comparison
34 between the contact angles measured before and after the exposure to PBS clearly points out that
35 a decrease in the surface energy of PEDOT:PSS is induced by the interaction with the electrolyte
36 solution, irrespectively to the applied voltage (Table 3). This effect can be explained by
37 considering that PSS in excess is partially removed when the films are immersed in water.³⁰
38 Excess PSS is expected to be mostly accumulated on the surface of the films,³¹ and because of
39 the presence of HSO₃⁻ groups, it can be dissolved by water thanks to the formation of hydrogen
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

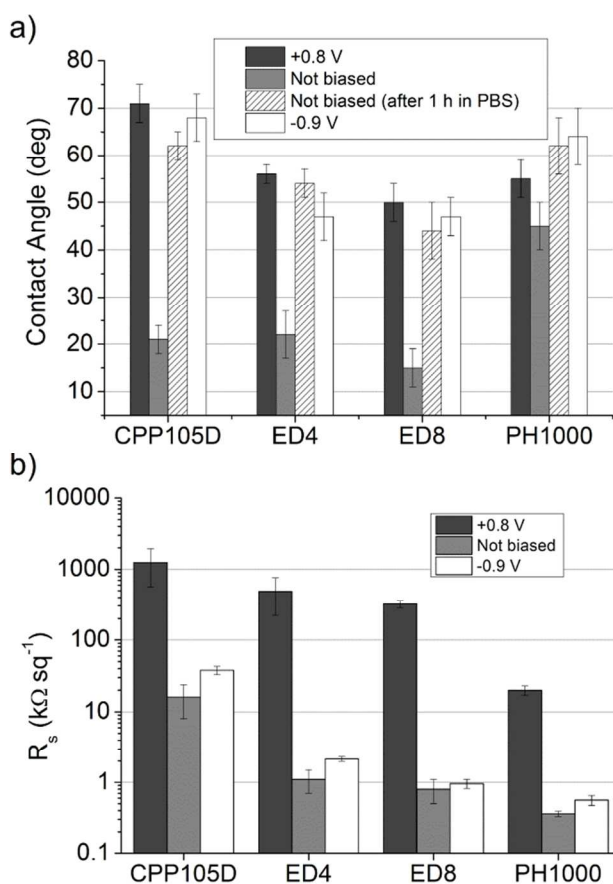
1
2
3 bonds, making the surface of the film hydrophilic. Since the PSS in excess is poorly interacting
4 with PEDOT, its removal in water has no significant effect on the electrical and electrochemical
5 properties of PEDOT:PSS. On the other hand, a change in the oxidation state of PEDOT:PSS
6 does not seem to influence the hydrophilicity and the surface energy of this material, conversely
7 to what was observed for instance on PEDOT:Tosylate.⁵ Thanks to their relatively small
8 dimensions compared to PSS (171 vs. 70,000 Da), tosylate anions can be completely removed
9 from the polymer matrix and undergo ion exchange when immersed in an electrolyte solution,³²
10 while this effect is hindered for PSS due to steric effects. As a consequence, the exchange of PSS
11 anions is mechanically impeded, as well as the switching of charged sulfonate groups from
12 PEDOT to the surface. This explanation is confirmed by the fact that PEDOT:PSS exchanges
13 cations (Na^+ , K^+ , etc.) with the electrolyte solution to maintain electrical neutrality,³³ while the
14 polymer exchanges its counterion when small dopant molecules, like tosylate or perchlorate, are
15 used.³² Moreover, since the outer part of the film is mainly composed by an excess of PSS, the
16 variation of the redox state of inner PEDOT does not affect the surface processes. As a result, the
17 surface energy of the films is weakly influenced, if not at all, by the applied polarization, even if
18 charge distribution in the PEDOT cores is effectively modified.
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39

40 These results suggest that, regarding PEDOT:PSS, surface energy and wettability cannot be
41 considered key parameters for controlling cell growth.
42
43
44

45 **3.3 Sheet resistance**

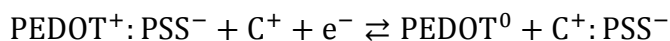
46 The sheet resistance (R_s) of PEDOT:PSS thin films was measured using a 4-probe setup before
47 and after the application of a redox voltage to the samples for 1 h. The results are reported in
48 Figure 5b. PH1000 films have the lowest sheet resistance (R_s about 0.36 k Ω /sq). ED4 and ED8
49 show slightly higher values (R_s about 1.1 and 0.8 k Ω /sq, respectively), but still comparable,
50
51
52
53
54
55
56
57
58
59
60

1
2
3 while the sheet resistance of CPP105D is at least ten times higher compared to the other
4 materials. This high difference is partially due to the absence of the cross-linking agent (GOPS),
5 which is proven to affect PEDOT:PSS conductivity,³⁰ in the CPP105D suspension used as
6 substrate for electropolymerization. Indeed, the presence of GOPS 1 v/v% was found to increase
7 the surface resistance of CPP105D and PH1000 by a factor of 2.
8
9
10
11
12
13
14
15
16
17
18



19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47 **Figure 5.** a) Water static contact angle measured on the PEDOT:PSS samples under study in
48 their oxidized, not biased and reduced form. The not biased samples were measured as deposited
49 and after being immersed in PBS for 1 h. b) Sheet resistance measured on all the four different
50 sets of PEDOT:PSS samples here investigated, in their oxidized, not biased and reduced form.
51
52
53
54
55
56
57
58
59
60

1
2
3 The effect of the electrochemical redox reaction on the conductivity of PEDOT:PSS depends
4 on the polarization (anodic or cathodic) of the voltage applied to the electrode. When a reducing
5 (i.e. negative) potential is applied to the PEDOT:PSS electrode, the following reaction occurs:³³
6
7



8
9
10
11
12 Where C^+ and e^- are, respectively, cations and electrons within the electrolyte solution. Hence,
13 PEDOT changes from its oxidized (conductive) state to its neutral (not conductive) state, and an
14 increase in the sheet resistance is observed. When an oxidizing (i.e. positive) voltage is applied
15 to the PEDOT:PSS electrode, the polymer film changes from its pristine partially-oxidized state
16 to a more conductive fully-oxidized state. However, if the positive bias is over 0.6 V,
17 PEDOT:PSS undergoes overoxidation, which induces a dramatic increase in electrical resistance,
18 as observed in all our tested samples. It is noteworthy that, despite the higher carrier density
19 measured in the electrodeposited samples (see Paragraph 3.1), PH1000 shows the highest
20 electrical conductivity, indicating that in PH1000 the charge carriers have a much larger mobility
21 that compensates their lower density. Finally, the effect of the exposure to cell culture medium
22 (DMEM) was tested on not biased samples for up to 48 h, finding that the surface resistance
23 increases by about a factor of two in this time period (see Supporting Information). This change
24 mostly takes place within the first hour, indicating that is probably due to a spontaneous
25 reorganization of the polymer chains induced by the interaction with the electrolyte (which
26 lowers carrier mobility) rather than a degradation effect.
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

48 By selecting the redox potentials reported in Figure 5b we can thus obtain PEDOT:PSS thin
49 films with different yet controlled electrical transport properties, one of the key parameters
50 whose role has to be assessed when PEDOT:PSS is used as a cell growth substrate.
51
52
53
54
55
56
57
58
59
60

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,

1
2
3 irrespective of the type of film used (Figure 6b and 7). Furthermore, within the
4
5 experimental errors, the T98G proliferation rate is the same on all the not biased
6
7 PEDOT:PSS substrates, with the only exception of ED4.
8
9

10 As indicated by the physical parameters of PEDOT:PSS, the techniques used for the film
11
12 depositions create surfaces characterized by different RMS roughness, thickness, surface energy
13
14 and wettability. Differently, the proliferation rate analysis, for both normal and tumor cells, did
15
16 not show significant differences between the four types of pristine substrates (Supporting
17
18 Information), therefore indicating that the morphology of the PEDOT:PSS surfaces does not
19
20 influence the growing capability of these cells.
21
22
23

24 It is interesting to note that even if the hydrophilicity of the PH1000 pristine form is
25
26 significantly lower compared to CPP105D-based films, also in this case, the proliferation rate of
27
28 both cells population is not affected.
29
30

31 From the data acquired we can also observe that the application of an external bias able to
32
33 change the redox state of the polymer does not affect surface morphology, energy and
34
35 hydrophilicity with respect to the pristine one; while the sheet resistance is strongly increased
36
37 only by the application of an oxidizing potential in all substrates. Nevertheless, since the
38
39 proliferation rates are not modified by any of the oxidized substrates except for the hDF growing
40
41 on PH1000, we can exclude a causal relationship between sheet resistance and proliferation
42
43 capability on biased PEDOT:PSS. On the basis of these evidences, it seems that the
44
45 electrochemical status is an independent perturbing key factor able to induce a detectable
46
47 modification of biological systems like the one used. The dependency of cell response to the
48
49 applied bias used to reduce the PEDOT films was preliminarily assessed by growing T98G cells
50
51 on ED4 substrates that had been biased at -0.4 V. While the difference of normalized cell number
52
53
54
55
56
57
58
59
60

at 72 h between not-biased and films reduced at -0.9 V is 1.3 ± 0.4 , in the case of films reduced at -0.4 V it drops to 0.3 ± 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.

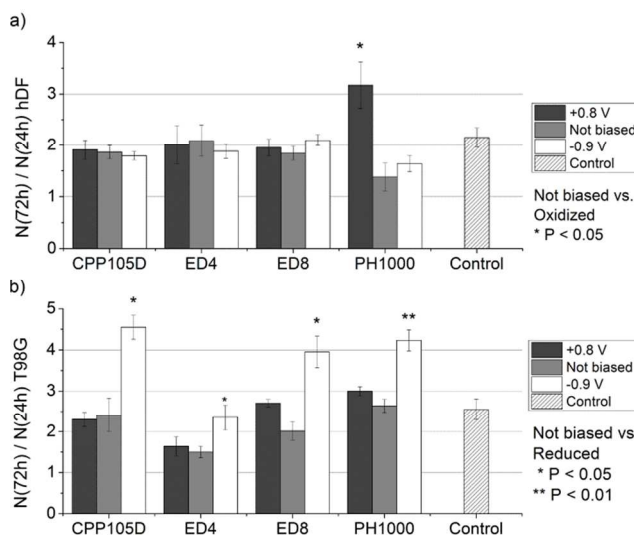


Figure 6. Effect, after 72 hours from seeding, on the proliferation rate of the PEDOT:PSS substrates altered by an oxidation ($+0.8\text{ V}$) or reduction (-0.9 V) process. a) Proliferation rate of hDF cells plated on not biased PEDOT:PSS CPP105D ($n=4$), ED4 ($n=4$), ED8 ($n=6$), PH1000 ($n=4$) and 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.

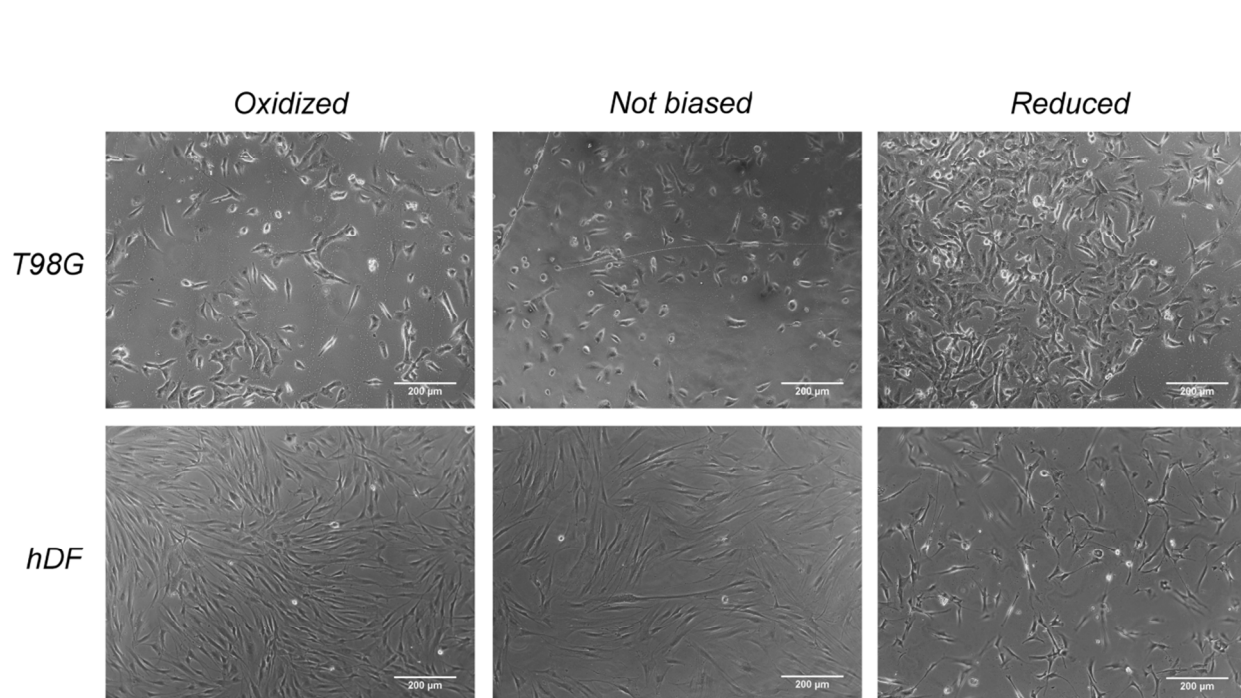
Furthermore, the interesting finding that hDF proliferation rate increases only when seeded on oxidized PH1000, while the reduced form of any PEDOT:PSS type tested is able to induce an

1
2
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.
5
6

7
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^{10,11,34} affecting adhesion and growth^{35,36} of very
16 sensitive cells such as primary hDF.
17
18
19
20
21
22
23
24
25
26
27

28
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,³⁷ our results might be interpreted as a different effect of the reduced substrate on the
37 integrins ligation status.
38
39
40
41
42
43
44
45
46
47
48
49

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.
52
53
54
55
56
57
58
59
60



25
26
27
28
29
30
31
32

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 μ m.

33 34 35

4. Conclusion

36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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).

1
2
3 Electrochemical analyses showed that the oxidized (i.e. positively biased) form of
4 PEDOT:PSS is more stable in time than the reduced (i.e. negatively biased) form when exposed
5 to an aqueous medium, and a redox potential between -0.2 V and +0.6 V was found to give a
6 very reproducible response in cyclic voltammetries. However, higher potentials (-0.9 V and +0.8
7 V) were used in order to maximize the differences between reduced, not biased and oxidized
8 forms of PEDOT:PSS both in terms of physical properties and influence on cell growth.
9

10
11 Surface morphology of PEDOT:PSS films was found to be affected by the deposition
12 technique used. Surface roughness ranged from 6 nm for CPP105D to 33 nm for ED8, increasing
13 with an increasing number of electropolymerization cycles. On the other hand, the redox state of
14 the polymer did not affect its surface morphology.
15

16
17 Static water contact angle measurements showed that wettability and surface energy
18 significantly decreased when PEDOT:PSS interacted with PBS, while no significant change was
19 induced by the application of an external bias.
20

21
22 Sheet resistance was strongly affected by the application of an oxidizing potential, which
23 induced an increase in the electrical resistance of about two orders of magnitude in all the
24 samples observed. The exposure to cell culture medium for 48 h induced an increase of the sheet
25 resistance by a factor of two in all the types of PEDOT:PSS.
26

27
28 Cell growth experiments were carried out to assess the effectiveness of the here studied four
29 different types of PEDOT:PSS films as substrates for controlled cell growth applications. We
30 used two different cell lines, primary human dermal fibroblasts (hDF) and glioblastoma
31 multiforme cells (T98G). The cells were seeded on reduced, not biased and oxidized
32 PEDOT:PSS thin films, and the effect of the redox status on cell growth was assessed after 72 h.
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 The result was found to be strongly cell-dependent, suggesting that each cell type has its own
4 peculiar response to the same environment:
5
6

7
8 hDF proliferation rate was significantly enhanced specifically by oxidized PH1000, the only
9 substrate that we have observed to strongly exchange charge with the cell culture medium.
10
11

12
13 T98G cells proliferation rate was significantly enhanced on reduced samples, irrespective of
14 the material used as substrate.
15
16

17
18 These results indicate that the cells proliferation rate has a clear dependence on the
19 electrochemical state of its substrate, while it is not affected by the other parameters as surface
20 roughness, surface conductivity and surface energy, that are very similar between not biased and
21 reduced/oxidized PEDOT:PSS. As a confirmation, no significant difference in the cell growth
22 rate is observed between the not biased forms of the four types of PEDOT:PSS films here used
23 as substrates, even if they show different surface parameters.
24
25
26
27
28
29
30

31
32 The results presented in this work allow to gain a better understanding on the major physical
33 and electrochemical properties of PEDOT:PSS, and how they are influenced by the interaction
34 with aqueous environments and by the application of a redox voltage. Furthermore, we
35 highlighted the importance of a precise control in the substrate deposition and oxidation
36 parameters and how the substrate properties are capable to drive cells activity, like growth,
37 taking into account the peculiar features of each type of cell. A systematic study of conjugated
38 polymers and their interactions with living cells is crucial to bring order into the current
39 scattering of the results presented in recent literature (Table 1), so as to allow the development of
40 new applications that can take advantage of the favorable properties of these materials.
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 ASSOCIATED CONTENT
4
5

6
7 **Supporting Information.**
8

9
10 Cyclic voltammeteries at different scan rates of PEDOT:PSS; absorption spectra of ED4 taken at
11 different times after polarization; trends over time of the intensity of the absorption peaks of
12 reduced and oxidized PEDOT:PSS in different electrolytes; percentages of residual PEDOT in
13 the reduced and oxidized forms of PEDOT:PSS after one-hour dipping in different electrolytes;
14 absorption spectra of reduced, not biased and oxidized PEDOT:PSS after 48 h of immersion in
15 cell culture medium; sheet resistance of PEDOT:PSS oxidized using different redox biases and
16 its trend over time for up to 48 h in cell culture medium; comparison between growth rate of hDF
17 and T98G cells on control Petri dish and not biased PEDOT:PSS over time. This material is
18 available free of charge via the Internet at <http://pubs.acs.org>.
19
20
21
22
23
24
25
26
27
28
29
30
31

32 AUTHOR INFORMATION
33

34
35 **Corresponding Author**
36

37
38 *Email: marco.marzocchi4@unibo.it
39
40

41 **Author Contributions**
42

43
44 The manuscript was written through contributions of all authors. All authors have given approval
45 to the final version of the manuscript.
46
47
48

49 **Notes**
50

51
52 The authors declare no competing financial interest.
53
54
55
56
57
58
59
60

ACKNOWLEDGMENT

The authors acknowledge financial support by the Italian Research Ministry, under the project PRIN 2010-2011 “MIND”.

REFERENCES

[1] Nikolou, M.; Malliaras, G. G. Applications of Poly(3,4-ethylene-dioxythiophene) Doped with Poly(styrene sulfonic acid) Transistors in Chemical and Biological Sensors. *Chem. Rec.* **2008**, *8*, 13-22.

[2] Rivnay, J.; Owens, R. M.; Malliaras, G. G. The Rise of Organic Bioelectronics. *Chem. Mater.* **2014**, *26*, 679-685.

[3] Owens, R.; Malliaras, G. G. Organic Electronics at the Interface with Biology. *MRS Bull.* **2010**, *35*, 449-456.

[4] Wong, J. Y.; Langer, R.; Ingber, D. E. Electrically Conducting Polymers can Noninvasively Control the Shape and Growth of Mammalian Cells. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 3201-3204.

[5] Saltó, C.; Saindon, E.; Bolin, M.; Kanciurzewska, A.; Fahlman, M.; Jager, E. W. H.; Tengvall, P.; Arenas, E.; Berggren, M. Control of Neural Stem Cell Adhesion and Density by an Electronic Polymer Surface Switch. *Langmuir* **2008**, *24*, 14133-14138.

[6] Svennersten, K.; Bolin, M. H.; Jager, E. W.; Berggren, M.; Richter-Dahlfors, A. Electrochemical Modulation of Epithelia Formation using Conducting Polymers. *Biomaterials* **2009**, *30*, 6257-6264.

1
2
3 [7] Wan, A. M. D.; Brooks, D. J.; Gumus, A.; Fischbach, C.; Malliaras, G. G. Electrical
4 Control of Cell Density Gradients on a Conducting Polymer Surface. *Chem. Commun.* **2009**,
5 5278-5280.
6
7

8
9
10 [8] Greco, F.; Fujie, T.; Ricotti, L.; Taccola, S.; Mazzolai, B.; Mattoli, V. Microwrinkled
11 Conducting Polymer Interface for Anisotropic Multicellular Alignment. *ACS Appl. Mater.*
12 *Interfaces* **2013**, *5*, 573-584.
13
14
15

16 [9] Sivaraman, K. M.; Özkale, B.; Ergeneman, O.; Lühmann, T.; Fortunato, G.; Zeeshan, M.
17 A.; Nelson, B. J.; Pané, S. Redox Cycling for Passive Modification of Polypyrrole Surface
18 Properties: Effects on Cell Adhesion and Proliferation. *Adv. Healthcare Mater.* **2013**, *2*, 591-598.
19
20
21
22
23
24
25

26 [10] Wan, A. M. D.; Schur, R. M.; Ober, C. K.; Fischbach, C.; Gourdon, D.; Malliaras, G. G.
27 Electrical Control of Protein Conformation. *Adv. Mater.* **2012**, *24*, 2501-2505.
28
29
30
31

32 [11] Gelmi, A.; Higgins, M.; Wallace, G. Resolving Sub-Molecular Binding and Electrical
33 Switching Mechanisms of Single Proteins at Electroactive Conducting Polymers. *Small* **2013**, *9*,
34 393-401.
35
36
37
38
39

40 [12] Bettinger, C. J.; Langer, R.; Borenstein, J. T. Engineering Substrate Topography at the
41 Micro- and Nanoscale to Control Cell Function. *Angew. Chem. Int. Ed.* **2009**, *48*, 5406-5415.
42
43
44
45

46 [13] Tonazzini, I.; Bystrenova, E.; Chelli, B.; Greco, P.; Stoliar, P.; Calò, A.; Lazar, A.;
47 Borgatti, F.; D'Angelo, P.; Martini, C.; Biscarini, F. Multiscale Morphology of Organic
48 Semiconductor Thin Films Controls the Adhesion and Viability of Human Neural Cells. *Biophys.*
49 *J.* **2010**, *98*, 2804-2812.
50
51
52
53
54
55
56
57
58
59
60

1
2
3 [14] Baek, S.; Green, R. A.; Poole-Warren, L. A. The Biological and Electrical Trade-Offs
4 Related to the Thickness of Conducting Polymers for Neural Applications. *Acta Biomater.* **2014**,
5
6 *10*, 3048-3058.
7
8

9
10
11 [15] Lu, C. H.; Hsiao, Y. S.; Kuo, C. W.; Chen, P. Electrically Tunable Organic
12 Bioelectronics for Spatial and Temporal Manipulation of Neuron-Like Pheochromocytoma (PC-
13
14 12) Cells. *Biochim. Biophys. Acta* **2013**, *1830*, 4321-4328.
15
16
17

18
19 [16] Pires, F.; Ferreira, Q.; Rodrigues, C. A. V.; Morgado, J.; Castelo Ferreira, F. Neural Stem
20 Cell Differentiation by Electrical Stimulation using a Cross-Linked PEDOT Substrate:
21 Expanding the Use of Biocompatible Conjugated Conductive Polymers for Neural Tissue
22 Engineering. *Biochim. Biophys. Acta* **2015**, *1850*, 1158-1168.
23
24
25
26
27

28
29 [17] Strakosas, X.; Bongo, M.; Owens, R. M. The Organic Electrochemical Transistor for
30 Biological Applications. *J. Appl. Polym. Sci.* **2015**, *132*, 41735.
31
32
33

34
35 [18] Smela, E. Conjugated Polymer Actuators. *MRS Bull.* **2008**, *33*, 197-204.
36
37

38 [19] Baran, D.; Balan, A.; Celebi, S.; Meana Esteban, B.; Neugebauer, H.; Sariciftci, N. S.;
39 Toppare, L. Processable Multipurpose Conjugated Polymer for Electrochromic and Photovoltaic
40 Applications. *Chem. Mater.* **2010**, *22*, 2978-2987.
41
42
43
44

45
46 [20] Otero, T. F.; Martinez, J. G.; Arias-Pardilla, J. Biomimetic Electrochemistry from
47 Conducting Polymers. A Review: Artificial Muscles, Smart Membranes, Smart Drug Delivery
48 and Computer/Neuron Interfaces. *Electrochim. Acta* **2012**, *84*, 112-128.
49
50
51
52

53
54 [21] Chibowski, E.; Perea-Carpio, R. Problems of Contact Angle and Solid Surface Free
55 Energy Determination. *Adv. Colloid Interface Sci.* **2002**, *98*, 245-264.
56
57
58
59
60

1
2
3 [22] Sze, S. M.; Ng, Kwok K. *Physics of Semiconductor Devices*; Wiley: Hoboken, NJ, 2007.

4
5
6 [23] Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*;
7
8 Wiley: Hoboken, NJ, 2000.

9
10
11 [24] Skotheim, T. A.; Reynolds, J. R. *Conjugated Polymers: Theory, Synthesis, Properties,*
12
13 *and Characterization*; CRC Press: New York, NY, 2007.

14
15
16 [25] Chen, X.; Inganäs, O. Three-Step Redox in Polythiophenes: Evidence from
17
18 Electrochemistry at an Ultramicroelectrode. *J. Phys. Chem.* **1996**, *100*, 15202-15206.

19
20
21 [26] Massonnet, N.; Carella, A.; Jaudouin, O.; Rannou, P.; Laval, G.; Cella, C.; Simonato, J.
22
23 Improvement of the Seebeck Coefficient of PEDOT: PSS by Chemical Reduction Combined
24
25 with a Novel Method for its Transfer using Free-Standing Thin Films. *J. Mater. Chem. C* **2014**,
26
27 *2*, 1278-1283.

28
29
30 [27] Elschner, A.; Kirchmeyer, S.; Lövenich, W.; Merker, U.; Reuter, K. *PEDOT: Principles*
31
32 *and Applications of an Intrinsically Conductive Polymer*; CRC Press: New York, NY, 2011.

33
34
35 [28] Garreau, S.; Duvail, J. L.; Louarn, G. Spectroelectrochemical Studies of Poly(3,4-
36
37 ethylenedioxythiophene) in Aqueous Medium. *Synth. Met.* **2002**, *125*, 325-329.

38
39
40 [29] Kvarnström, C.; Neugebauer, H.; Blomquist, S.; Ahonen, H. J.; Kankare, J.; Ivaska, A. In
41
42 Situ Spectroelectrochemical Characterization of Poly(3,4-ethylenedioxythiophene). *Electrochim.*
43
44 *Acta* **1999**, *44*, 2739-2750.

45
46
47 [30] Zhang, S.; Kumar, P.; Nouas, A. S.; Fontaine, L.; Tang, H.; Cicoira, F. Solvent-Induced
48
49 Changes in PEDOT:PSS Films for Organic Electrochemical Transistors. *APL Mater.* **2015**, *3*,
50
51 014911-4.

1
2
3 [31] Greczynski, G.; Kugler, T.; Salaneck, W. R. Characterization of the PEDOT-PSS System
4 by means of X-ray and Ultraviolet Photoelectron Spectroscopy. *Thin Solid Films* **1999**, *354*,
5 129–135.
6
7
8

9
10
11 [32] Hillman, A. R.; Daisley, S. J.; Bruckenstein, S. Kinetics and Mechanism of the
12 Electrochemical p-Doping of PEDOT. *Electrochem. Commun.* **2007**, *9*, 1316-1322.
13
14

15
16
17 [33] Nilsson, D.; Robinson, N.; Berggren, M.; Forchheimer, R. Electrochemical Logic
18 Circuits. *Adv. Mater.* **2005**, *17*, 353-358.
19
20

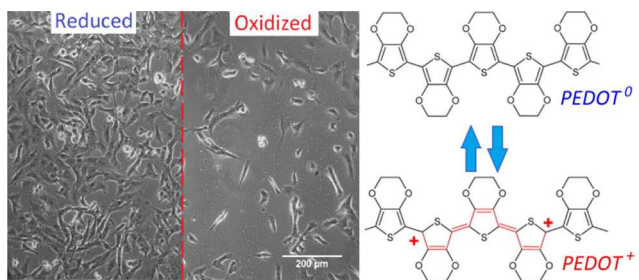
21
22 [34] Williams, E. C.; Janmey, P. A.; Ferry, J. D.; Mosher, F. D. Conformational States of
23 Fibronectin. Effects of pH, Ionic Strength, and Collagen Binding. *J. Biol. Chem.* **1982**, *257*,
24 14973-14978.
25
26
27

28
29
30 [35] Wan, A. M. D.; Chandler, E. M.; Madhavan, M.; Infanger, D. W.; Ober, C. K.; Gourdon,
31 D.; Malliaras, G. G.; Fischbach, C. Fibronectin Conformation Regulates the Proangiogenic
32 Capability of Tumor-Associated Adipogenic Stromal Cells. *Biochim. Biophys. Acta* **2013**, *1830*,
33 4314-4320.
34
35
36

37
38 [36] Keselowsky, B. G.; Collard, D.M.; García, A. J. Surface Chemistry Modulates
39 Fibronectin Conformation and Directs Integrin Binding and Specificity to Control Cell
40 Adhesion. *J. Biomed. Mater. Res. Part A* **2003**, *66A*, 247-259.
41
42
43

44
45 [37] Desgrosellier, J. S.; Cheresch, D. A. Integrins in Cancer: Biological Implications and
46 Therapeutic Opportunities. *Nat. Rev. Cancer* **2010**, *10*, 9-22.
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table of Contents Graphic



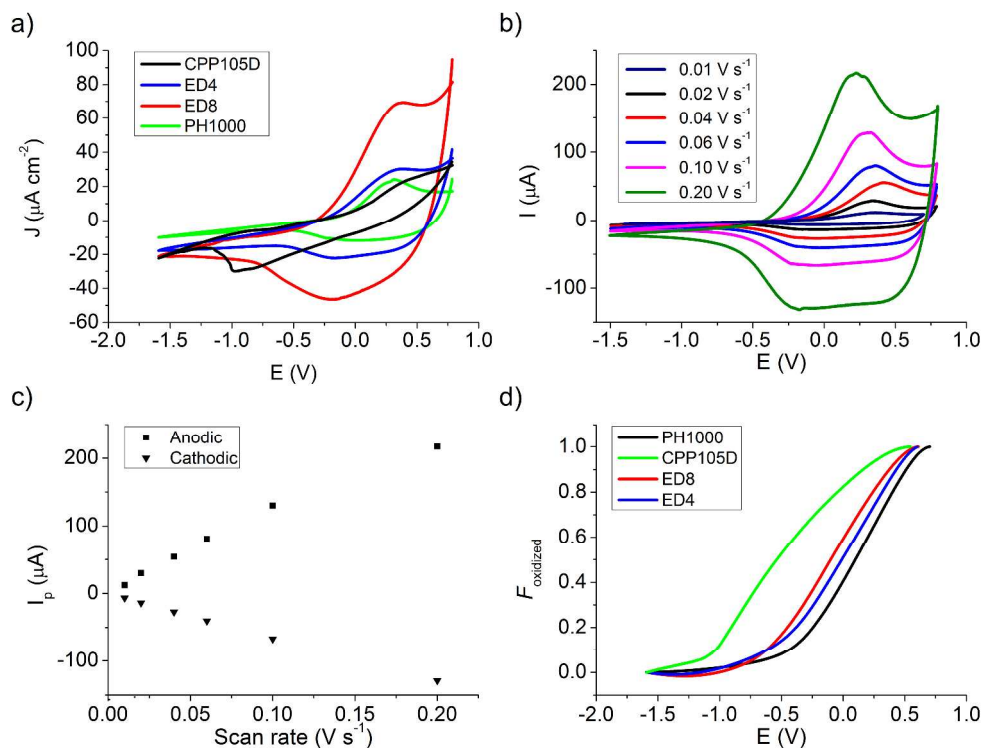


Figure 1 a) CVs recorded for different PEDOT:PSS substrates (scan rate 20 mV s⁻¹). b) Cyclic voltammograms of PH1000 taken at different scan rates. c) Peak current vs. scan rate. d) Fraction of oxidized PEDOT:PSS (F_{oxidized}) for the different substrates as a function of the applied voltage. All the graphs refer to measurements carried out in PBS
532x399mm (300 x 300 DPI)

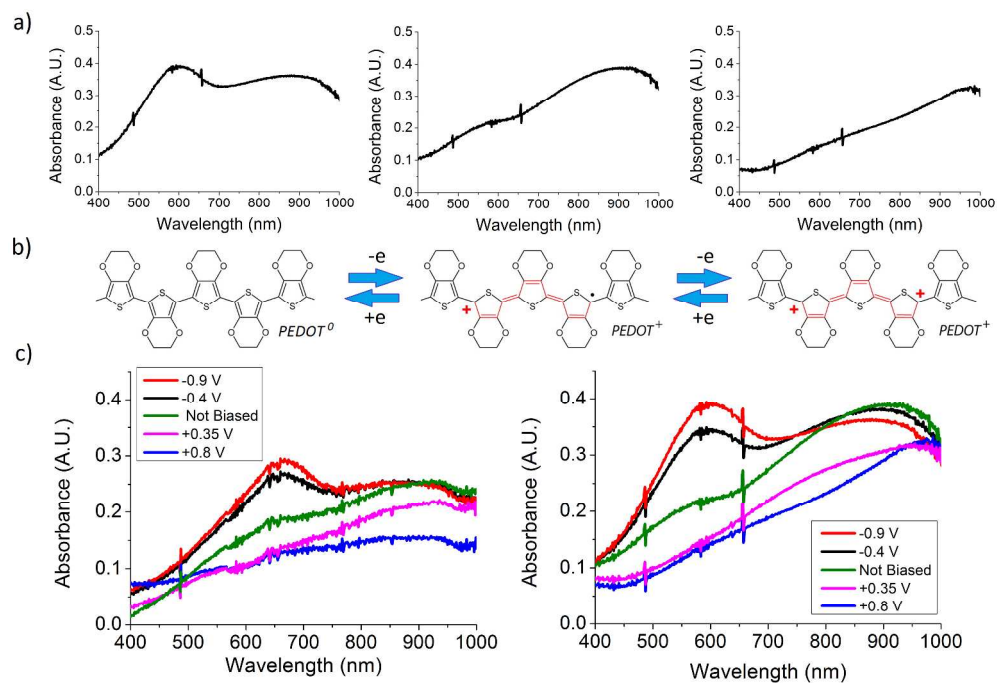


Figure 2 a) Visible and NIR spectra of ED8 in its reduced (-0.9 V, left), not-biased (middle) and oxidized (+0.8 V, right) forms. b) Schematics of the redox process in PEDOT from its reduced (left) to its fully oxidized (right) state. c) Visible and near IR spectra of PH1000 (left) and ED8 (right) in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

409x289mm (300 x 300 DPI)

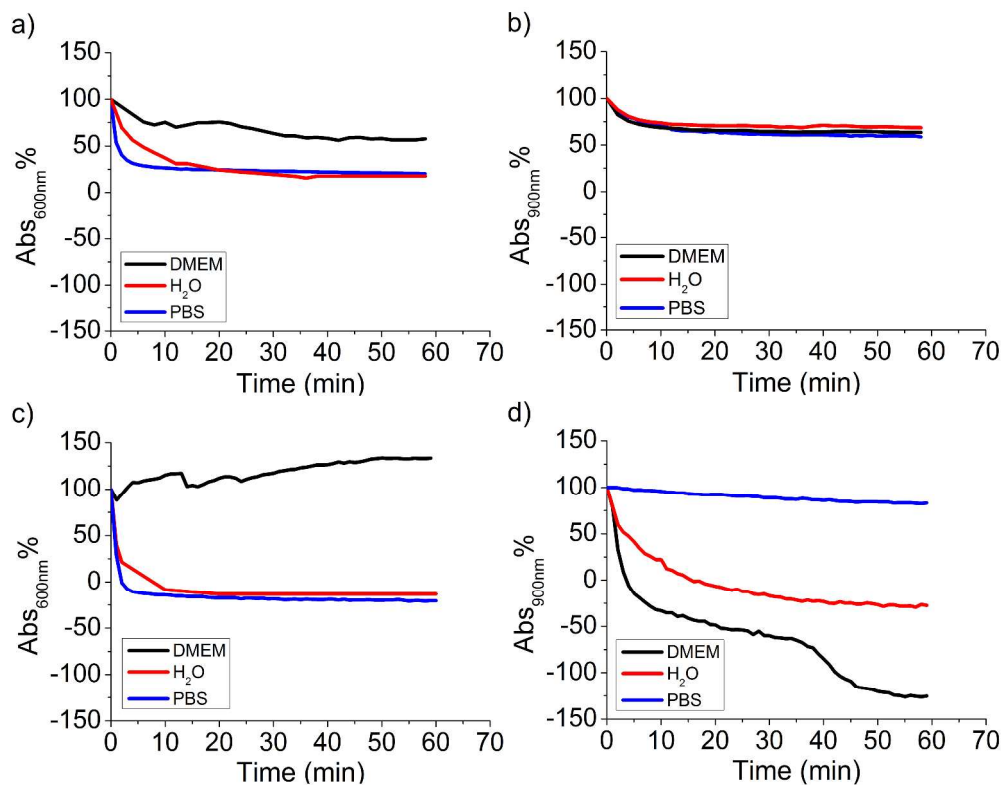


Figure 3 Trends of $Abs_{600nm}\%$ and $Abs_{900nm}\%$ for (a) reduced ED4, (b) oxidized ED4, (c) reduced PH1000, and (d) oxidized PH1000. The samples were biased at -0.9 and $+0.8$ V, and then immersed in DMEM, H₂O and PBS. The trends for CPP105D and ED8 are available in the Supporting Information.
520x403mm (300 x 300 DPI)

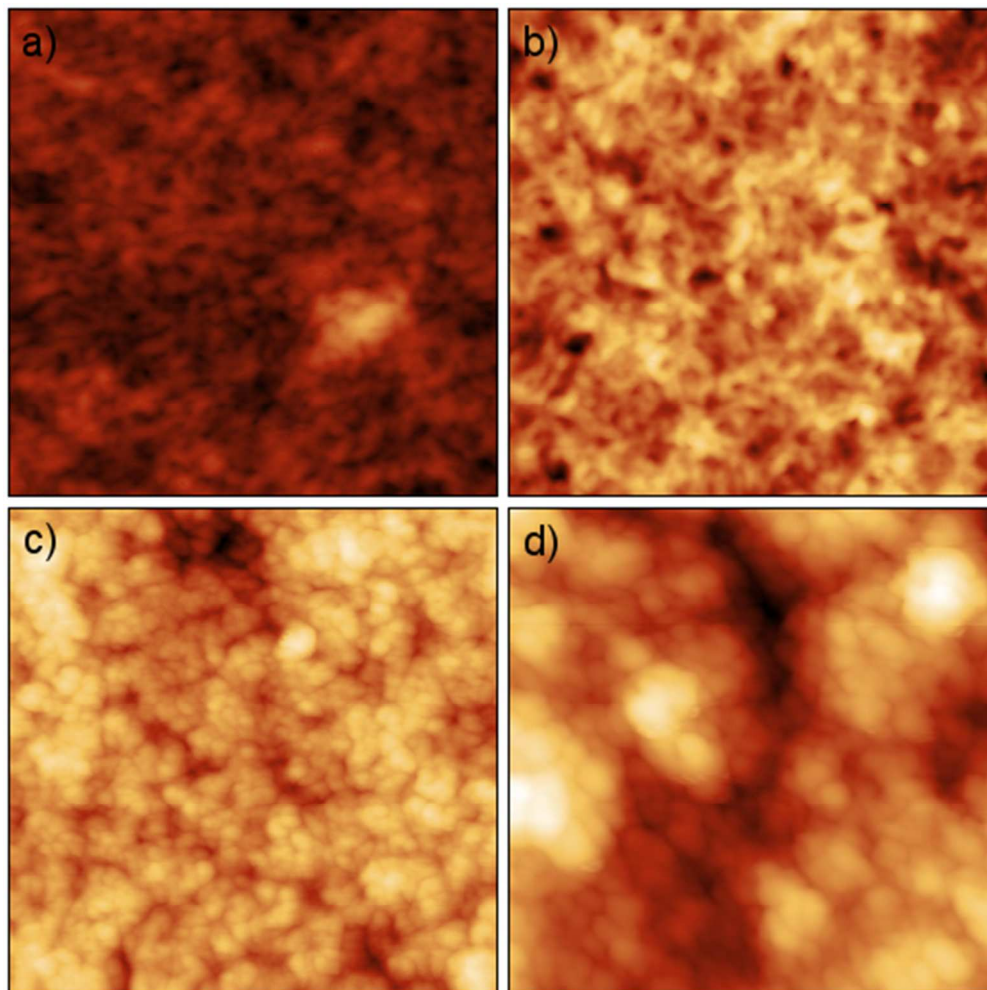


Figure 4 AFM topography images of the PEDOT:PSS samples under study: a) PH1000, b) CPP105D, c) ED4, d) ED8. Scan size: $1\mu\text{m} \times 1\mu\text{m}$, z scale: 35 nm for (a), (b) and (c), 90 nm for (d).
44x44mm (300 x 300 DPI)

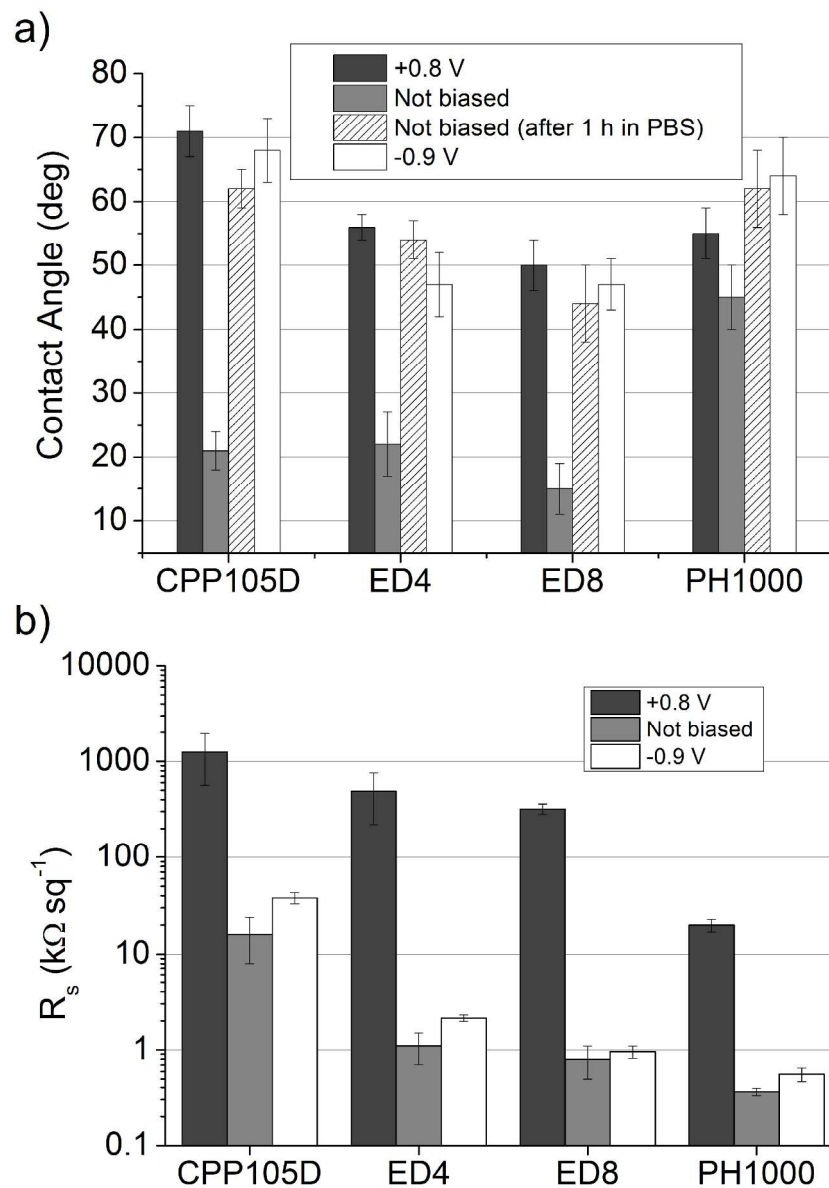
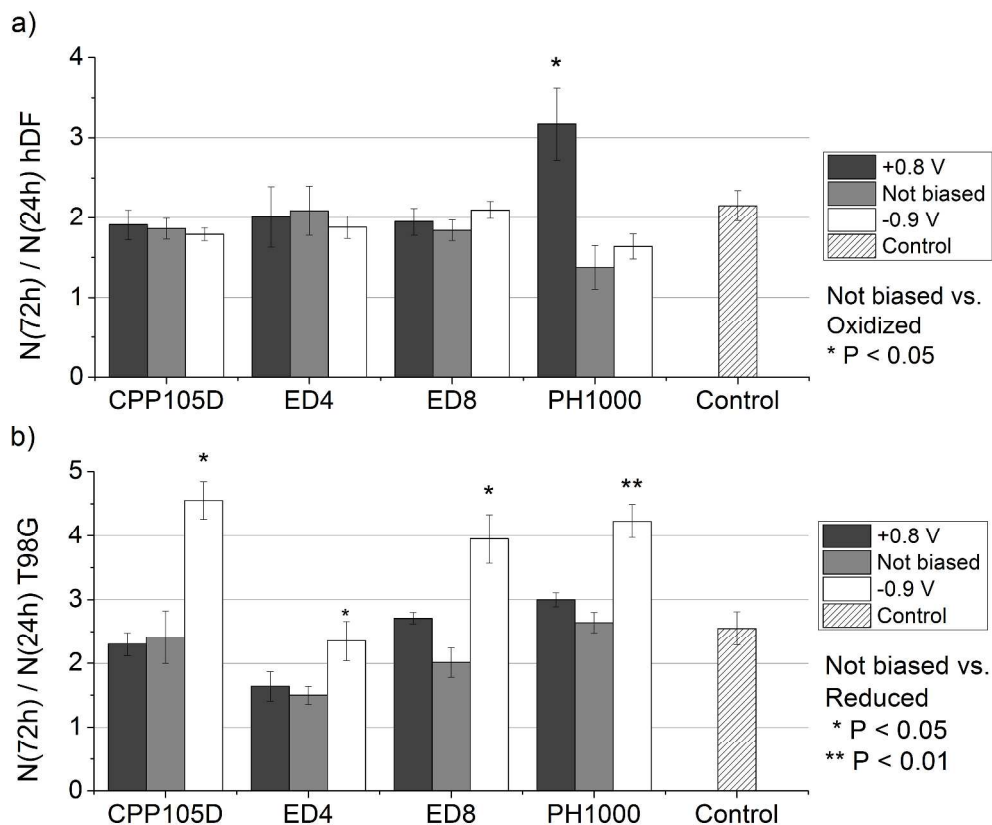
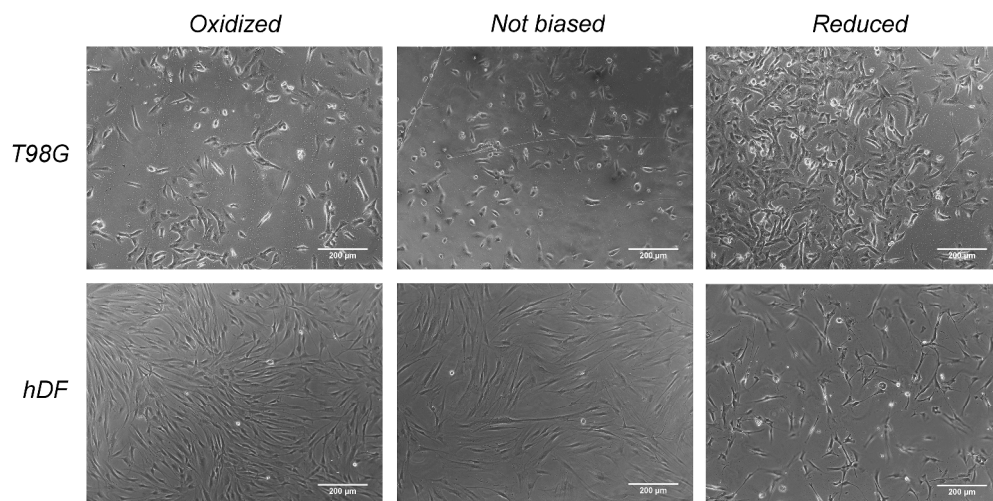


Figure 5 a) Water static contact angle measured on the PEDOT:PSS samples under study in their oxidized, not biased and reduced form. The not biased samples were measured as deposited and after being immersed in PBS for 1 h. b) Sheet resistance measured on all the four different sets of PEDOT:PSS samples here investigated, in their oxidized, not biased and reduced form.
202x282mm (300 x 300 DPI)



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

43 449x377mm (300 x 300 DPI)



24 **Figure 7** A representative example of T98G and hDF phase contrast microscopy images at 100× of
25 magnification, hDF and T98G proliferation on +0.8 V (oxidized), not biased and -0.9 V (reduced) PH1000
26 after 72 h from cell seeding. Scale bar 200 μm.
27 458x229mm (300 x 300 DPI)

28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60