Cell viability studies and operation in cellular culture medium of n-type organic field-effect transistors

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(Received 26 October 2010; accepted 4 January 2012; published online 13 February 2012)

The possibility of the fabrication of organic devices suitable to be applied in bio-sensing fields depends largely on the availability of organic compounds displaying robust electrical properties even in aqueous solutions and effective biocompatibility features. In this paper, we report about the good cellular biocompatibility and the electrical response stability in an ionic medium of n-type organic transistors based on the recently developed PDI-8CN_2 oligomer. The biocompatibility has been tested by analyzing the adhesion and viability of two different cell lines, human epithelial HeLa cells and murine neuronal F11 cells, on PDI-8CN_2 films grown by organic molecular beam deposition (OMBD) on SiO_2 substrates. The effect of film thickness on cell attachment was also tested. Uncoated SiO_2 substrates were used as control surfaces and sexithiophene (T6) as device testing control. Moreover, the possible toxicity of –CN groups of PDI-8CN_2 was tested on HeLa cell cultures, using PDI-8 and T6 molecules as controls. Results showed that, although at high concentration these organic compounds are toxic in solution, if they are presented in form of film, cell lines can attach and grow on them. The electrical response stability of PDI-8CN_2 transistors in a cellular culture medium characterized by high concentrations of ionic species has been also investigated. For this purpose, low-voltage operation devices with V_{GS} ranging from −5 V to 5 V, able to strongly reduce the influence of Faradaic currents coming from the electrical operation in an highly ionic environment, have been fabricated on 35 nm thick SiO_2 layers and electrically characterized. These results are useful to experimentally define the main critical issues to be further addressed for the fabrication of reliable bio-sensors based on organic transistors. © *2012 American Institute of Physics*. [doi:10.1063/1.3682109]

INTRODUCTION

Nowadays, the development of innovative materials for the realization of smart interfacing devices toward the biological systems is the subject of increasing attention by both industrial and academic research groups. The ultimate goal of this interest is the creation of new generations of disposable, portable, label-free, and highly sensitive devices to be easily and widely applied in diagnostics, health monitoring, and environmental agent control systems. Organic conjugated compounds, with their charge transport capabilities and visible light generation properties, are considered one of the most appealing material platforms to face these exciting challenges.[1] Indeed, owing to their mechanical properties and chemical composition, these "soft" materials can represent an ideal environment for the growth and proliferation of biological systems and for the simultaneous real-time monitoring via opto-electronic signals. From a technological viewpoint, organic (semi-)conducting films, where small (oligomers) or macro- (polymers) molecules are held together by weak van der Waals interactions, can be easily processed by low-cost, low temperature ($T < 200\ ^\circ C$) and large area deposition techniques on whatever type of substrate, even with transparent and flexible features. The electrical properties of these materials and their sensitivity to specific chemical and biological agents can be adequately tailored by the addition of selective reception groups to the basic molecular structure and/or by the fine tuning of the film features (i.e., microstructure, roughness).[2] Up to date, most efforts in this field have been focused on the class of conducting polymers (i.e., polypyrrole, PEDOT). These materials were showed to be highly compatible with the *in vitro* growth of cellular cultures and to be able in combining both electronic and ionic charge transport.[3] Sensing and/or stimulating electrodes both for *in vivo* and *in vitro* applications have been also fabricated. Furthermore, PEDOT films are currently employed in the realization of electrochemical transistors, where the conducting properties of a polymer channel can be externally modified by controlling its oxidation state.[4] Based on this phenomenon, large modulations (up to five orders of magnitude) of the active channel conductivity can be obtained, even if the dominant ionic transport limits the operation speed of these devices. Conversely, only few studies have been devoted so far to experimentally test if organic semiconducting compounds, usually displaying low conductivity values but significant charge carrier mobility, are actually suitable for the growth and proliferation of cell cultures or intact tissues. These materials are of pivotal importance for the fabrication of organic transistors (OFETs) which, based on the electrostatic modulation of the electronic charge carrier density, could be directly

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exploited in the detection/stimulation of bio-signals, since the active layer can operate both as sensitive area and charge transport channel. In this context, Bystrenova *et al*. reported on the growth of neuronal cells on evaporated pentacene films, showing that stem cells are able to differentiate into neurons and astrocytes, forming dense neuronal networks after two weeks.[5] The work of this group has been further developed and the influence of pentacene film morphology on the adhesion and viability of neural cells has been carefully addressed.[6] Very interestingly, these results prompt the idea that engineering substrates for cell research can be effectively pursued by the controlled growth of organic thin films. On the other hand, Scarpa *et al*. have investigated the viability of fibroblast human cells on poly(3-hexylthiophene) (P3HT) solution-processed films. In this case, it was shown that P3HT films need to be functionalized by the coverage of additional layers (i.e., collagene, fibronectin) to allow an acceptable rate of cell adhesion and proliferation.[7]

In order to give a further contribution to this emerging field, we present the results of biocompatibility cellular tests on organic films of *N,N′*–bis(n-octyl)-1,6-dicyanoperylene-3,4:9,10-bis(dicarboximide) (PDI-8CN_2 or Polyera ActivInk N1200), a very recently developed n-type (electron-transporting) compound.[8,9] We compared the biocompatibility of this material with sexithiophene (T6), an archetypal p-type (hole transporting) material, which has been studied for more than 20 years.[10] Perylene diimide compounds are presently the subject of an intense investigation, due to their ability to effectively carry electrons even in open-air conditions. Furthermore, their molecular structures can be finely tailored to get an accurate control of the final self-assembling properties and of the frontier orbital energetics. This last feature has a major impact on the thermodynamic stability of the charge transport in these compounds.[8] In our work, we have considered two cell culture types, analyzing both human epithelial (HeLa) cells and F11 rat dorsal root ganglion neuron X mouse neuroblastoma hybrid cells. To complete our study, the operational stability of PDI-8CN_2 transistors when immersed in extracellular culture medium has been investigated. In the perspective to develop n-type transistors of interest for bio-sensing applications, bottom-contact transistors with low-voltage operation have been fabricated and characterized.

MATERIALS AND METHODS

Growth of organic films and morphological properties

Sexithiophene (T6) and *N,N′*–bis(n-octyl)-1,6-dicyanoperylene-3,4:9,10-bis(dicarboximide) (PDI-8CN_2) powders were purchased by Sıgma-Aldrıch and Polyera Corporation, respectively. Bottom-contact bottom-gate transistors have been fabricated by joule evaporating PDI-8CN_2 and T6 films on suitable substrates, with Knudsen cells in a high-vacuum system (Pr$\sim 10^{-7}/10^{-8}$ mbar). Substrates consist in heavily n-doped silicon (Si^{++}), 200 or 35 nm thick SiO_2 thermally grown layers and interdigitated gold source-drain contacts. The utilized electrode configuration defines active channels with width (W) and length (L) of 22 mm and 40 μm, respectively. Before the deposition, substrates were cleaned by sonication in acetone and ethanol and used without any further chemical functionalization. In the standard deposition conditions, the evaporation rate was fixed at 1 nm/min and the entire deposition chamber was heated at 100 °C.[11,12] Film thickness was varied in the range between 10 and 30 nm. Detailed results about T6 and PDI-8CN_2 film morphology, also as a function of the film thickness, can be found in other reports.[13,14] Briefly, both T6 and PDI-8CN_2 films on SiO_2 surface display a polycrystalline island microstructure, where molecules lie with their long c-axis almost perpendicular to the substrate surface. This molecular packing is well known to strongly support the charge transport.[15] However, while in T6 films, grown with deposition parameters similar to those we used, the crystalline domains are basically disk-shaped (with possibly fractal contours), the crystallites in PDI-8CN_2 films are more elongated in a preferential direction with faceted walls and rounded corners. Similarly to other organic semiconducting oligomers deposited by joule evaporation, the growth dynamics of PDI-8CN_2 and T6 films exhibits a transition from a 2 D to a 3 D mode as a function of the thickness with increasing roughness. For about 30 nm thick films, the surface roughness ranges between 1 and 2 nm for both types of molecules.

Cell viability experiments

To study biocompatibility of organic films, two cell types have been used: (i) Epithelial HeLa cells, derived from human cervical cancer cells, which are widely used in biological research and (ii) F11 rat dorsal root ganglion neuron X mouse neuroblastoma hybrid cells,[16,17] which are particularly used as models of sensory neurons. Cells were plated and let to grow for 48 h onto the Si^{++}/SiO_2/Au (electrodes) substrates covered with T6 or PDI-8CN_2 films placed into standard 24-well cell culture plates (2×10^6 cells per well; each well had a surface area of 1.9 cm^2, substrate surface area: 1 cm^2; the seeding conditions were selected on the basis of pilot experiments to obtain good coverage of SiO_2 substrates after 1-2 days in culture). The substrates were previously sterilized using UV light for at least 30 min. Cells were also plated on Si^{++}/SiO_2/Au (electrodes) substrates without organic film coverage as controls for 48 h. HeLa cells were grown in RPMI 1640 (a standard culture medium originally developed at Roswell Park Memorial Institute) with L-glutamine, whereas F11 cells were grown in Dulbecco's modified medium (DMEM), supplemented with 2 mM glutamine and 10% fetal calf serum (FBS) plus 50 units/ml penicillin and 50 g/ml streptomycin in a humidified 5% CO_2 atmosphere at 37 °C.

To analyze cell shape and adhesion of cells onto substrates, cells were fixed with 4% paraformaldehyde for 10 min, washed in phosphate buffered saline (PBS) and then incubated with Hoechst 33258 and propidium iodide (PI) for 5 min to visualize cell nuclei and cell shape, respectively. After fixation, PI can enter into all cells, visualizing double-stranded DNA (in the nucleus) and RNA (in the cytoplasm), thereby giving strong fluorescent signal of the cell contours. After washing with PBS, stained cells were identified under a fluorescence microscope (Leica DMI3000), using excitation/emission wavelengths of 340/440 and 568/585 nm for

Hoechst 33 258 and PI, respectively. These fluorescent markers were selected due to the strong excitation and therefore minimal cross talk problems with the possible fluorescence emitted by the organic materials.

Ten randomly selected fields were acquired from each type of substrate on different places (e.g., onto interdigitated electrodes, on electrode free-areas, or on areas not covered by the organic material). The two channels were merged using Image J software. A minimum of 100 cells were manually counted for each substrate type. The number of cells attached on organic films was also normalized with respect to the number of cells on SiO_2, because the peripheral region of the substrate was free from the organic layer. Therefore, the number of cells grown on these peripheral areas represented an internal control for normal growing of the cells.

In separate experiments, to test the cytotoxicity of the organic materials, HeLa cells were seeded into 24-well plates and exposed for 3 h to either PDI-$8CN_2$ (0.5 mM, 50 μM, 5 μM) or T6 (1.5 mM, 0.15 mM, 15 μM, 1.5 μM) dissolved in dimethyl sulfoxide (DMSO) and directly added to the culture medium. In order to test specifically the toxicity contribution from –CN groups, additional tests were performed by using *N′*–bis(n-octyl)-perylene-3,4:9,10-bis(dicarboximide) (PDI-8, Sigma Aldrich, 1.35 mM, 0.135 mM, 13.5 μM, 1.35 μM).[18] Control wells were treated with equal amounts of DMSO vehicle. Subsequently, cells were fixed with 4% paraformaldehyde in PBS; transmitted light images were then acquired with an inverted microscope and the number of cells per high magnification field manually counted using Image J software.

To further characterize the possible cytotoxic effects of organic semiconductors, HeLa cells were cultured for 24 and 48 h on substrates covered by T6 and PDI-$8CN_2$ films as described above, and then stained using standard propidium iodide-fluorescein diacetate technique to count the number of living and dead cells. Propidium iodide- (PI; 70 μM) and fluorescein diacetate (FDA; 30 μM) positive cells were counted in ten random fields of independent cultures, and cell death was determined by the ratio of the number of PI-positive cells/FDA-stained-positive cells. Moreover, since the organic film does not cover the entire substrate, images were also sampled at the border between the organic layer and the SiO_2 surface. To identify the border of the organic layer the devices were observed using epi-illumination (Figs. 1(c), 1(d)). In this regard, Figs. 1(a) and 1(b) show a representative image of cells growing on the border of the organic layer.

Electrical characterization of PDI-$8CN_2$ transistors

The electrical response of as-fabricated PDI-$8CN_2$ transistors was analyzed in air and in dark conditions by using a Janis Probe station. The voltage-current signals were generated and collected by a picoammeter Keithley 487 and a digital multimeter Keithley 2400, connected to the probe-station arms through BNC connectors. To investigate the operation of PDI-$8CN_2$ devices in complex ionic media, a PDMS well was attached on the SiO_2 surface and filled with Dulbecco's modified medium (DMEM) in order to completely cover the device active channels. Then, the electrical response was recorded at different times of immersion. During the experiments, care was taken to keep a stable condition of device coverage by DMEM.

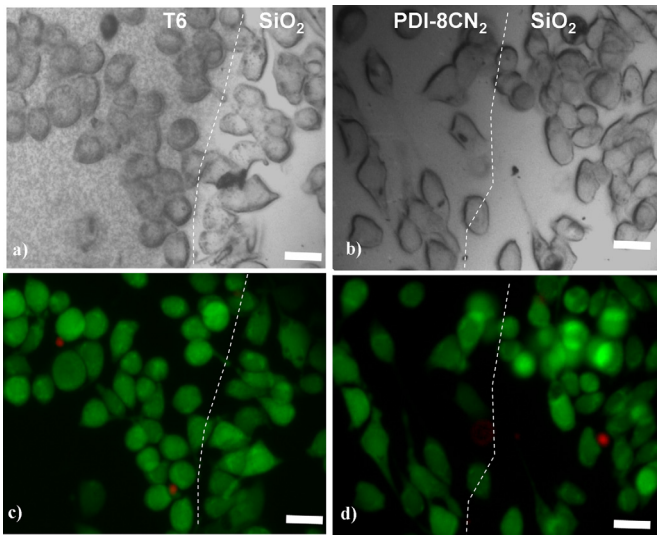

FIG. 1. (Color online) Distribution of HeLa on the border between the organic layer ((a), (c): T6; (b), (d): PDI-$8CN_2$) and SiO_2 uncovered (bare) surfaces. (a), (b): reflected light image; (c), (d): PI-FDA staining of the same field as in (a), (b). Scale-bar: 20 μM.

RESULTS AND DISCUSSION

Cell adhesion and viability analysis

Cell adhesion and toxicity assays were used to test the influence of PDI-$8CN_2$ on two different cell lines. F11 and HeLa cells, neuronal and epithelial cell lineages specifically, were selected as cellular models due to their large use in biological setups. Moreover, by comparison, the same tests were performed by using T6 films and uncovered (bare) SiO_2 substrates. Figure 2 represents a typical sparse, spindle-shaped F11 cells and polygonal confluent HeLa cells on SiO_2 substrates and on T6 and PDI-$8CN_2$ films (both 30 nm thick), 48 h after plating. Both cell types showed normal morphological features on SiO_2, T6 and PDI-$8CN_2$ substrates, thereby demonstrating their ability to adhere to these substrates and to survive in their presence (see also Fig. 6(d) for a comparison of the shape of HeLa cells grown on standard plastic substrates). The differences between F11 and HeLa cells in their ability to cover the substrate is due to the different biological properties of these two cell types: On plastic substrates, F11 cells do not form adherent junctions between their plasmalemmal membranes, thus remaining isolated and spontaneously forming cellular processes; moreover at high cellular densities they detach and die. Conversely, HeLa cells form junctions between their plasma membranes, thus forming cellular layers that can fill the entire substrate. It is interesting that in presence of the gold electrodes on the OFET device, cells had the tendency to adhere first to the region of the electrodes (data not shown) and then spread to the regions without gold electrodes. To this regard, it should be noted that the thickness of the

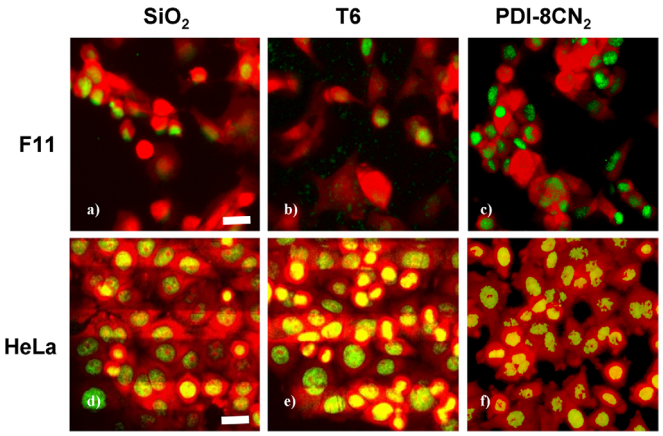

FIG. 2. (Color online) Typical morphology of F11 ((a), (b), (c)) and HeLa ((d), (e), (f)) cells grown on bare SiO_2 substrates, T6 and PDI-$8CN_2$ films, respectively. Scalebar: 20 μM.

organic layer is small if compared to the height of the electrode (100 nm), whereas cells have a height of about 5-8 μm and thickness larger than that of the electrode (see Fig. 1 for reference). Therefore, the behavior of the cells on gold electrodes may be due to this large "stair" on the surface of the organic layer.

Quantitative morphological analysis of F11 cells grown on SiO_2, T6, and PDI-$8CN_2$ surfaces revealed that cell adhesion was similar on these three different substrates (see Fig. 3). Conversely, HeLa cells cultured on PDI-$8CN_2$ were about 40% less abundant than on SiO_2 or T6 substrates. Indeed, separate one-way ANOVA for F11 cells and HeLa cells using SIO_2, T6, and PDI-$8CN_2$ as factors did not reveal significant differences for F11 cells on the three substrates ($SiO_2 = 260 \pm 54$ cells/mm^2; $T6 = 293 \pm 71$ cells/mm^2; PDI-$8CN_2 = 242 \pm 34$ cells/mm^2; $F = 0.31$, $p = 0.73$, one-way ANOVA test), whereas significant differences were found for HeLa cells ($SiO_2 = 470 \pm 82$ cells/mm^2; $T6 = 563 \pm 40$ cells/mm^2; PDI-$8CN_2 = 303 \pm 12$ cells/mm^2; $F = 25.12$, $p < 0.01$, one-way ANOVA). This contrasting behavior between F11 and HeLa cells may reflect the differences in the cell cycle between these two lines: F11 cells usually do not form a confluent cell layer and many cells can differentiate producing several neurites, whereas HeLa cells proliferate continuously until confluence. Therefore, to further understand the effect of PDI-$8CN_2$ on cell adhesion, we evaluated the attachment of F11 neuronal cells on PDI-$8CN_2$ films at different thickness (see Fig. 4, where normalized data are presented).

The number of F11 cells on 30 nm thick PDI-$8CN_2$ films was comparable to the number of cells adherent on bare SiO_2 substrates. More interestingly, the adhesion of F11 cells on PDI-$8CN_2$ appeared to increase for thinner films (SiO_2: 259 ± 55 cells/mm^2; PDI-$8CN_2$ 10 nm: 619 ± 55 cells/mm^2; PDI-$8CN_2$ 15 nm: 535 ± 83 cells/mm^2; PDI-$8CN_2$ 30 nm: 264 ± 35 cells/mm^2; $F = 5.961$, $p < 0.01$, one-way ANOVA). A similar dependence of the cellular attachment on film thickness was recently found also for human neural astrocytoma 1321N1 cells grown on pentacene films.[6] In our case, a possible explanation for this occurrence could be related to the

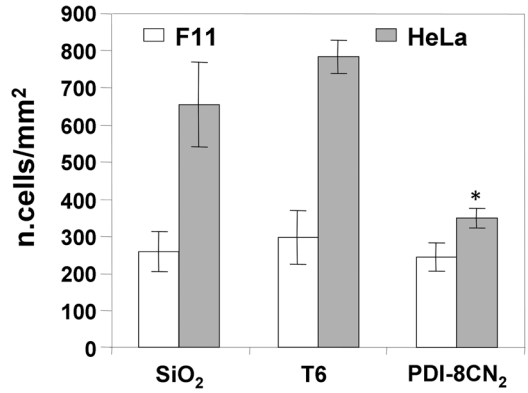

FIG. 3. HeLa and F11 cell adhesion on 30 nm thick T6 and PDI-$8CN_2$ films. Asterisk: $p < 0.05$ PDI-$8CN_2$ vs SiO_2 (LSD *post hoc* tests for multiple comparison).

increasing surface roughness when thicker films (where a three-dimensional growth mode is dominant) are considered.[14] However, it should be also noticed that, since only 30 nm thick films exhibit a significantly reduced cell adhesion levels, it is not possible to exclude that other effects (such as a partial surface degradation with material desorption) can be involved in the observed phenomenon. Future tests could be devoted to further clarify this phenomenon.

Finally, since HeLa cells showed reduced cellular density when grown on PDI-$8CN_2$, we tested if the organic material was actually toxic for cells with a high cell division rate (such as HeLa cells) using two approaches: (i) Adding in solution the organic compounds to a standard culture and evaluating the number of cells attached to the plastic substrate, and (ii) evaluating the number of dead cells grown on organic films using the PI/FDA technique. Therefore, we incubated HeLa cells with increasing concentrations of T6 and PDI-$8CN_2$ and studied the number of cells in culture after 3 h. Since the material was added to the cell medium on already attached cells, the changes should reflect only the toxicity of the material. As shown in Fig. 5, the results demonstrated that at high concentration of both organic materials, the number of cells adherent on the plastic substrate, normally used for cell cultures, was reduced. We further

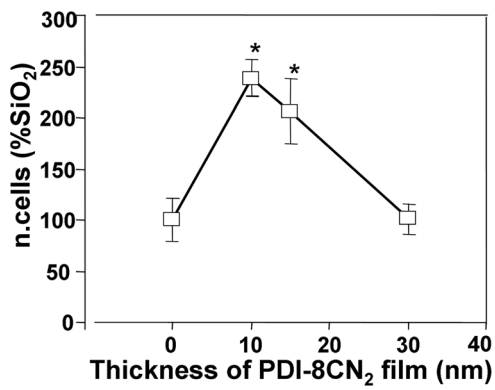

FIG. 4. F11 cell adhesion on PDI-$8CN_2$ films as a function of the film thickness. The values are normalized to those on bare SiO_2 substrates. Thickness = 0 refers to the absence of organic film (cells grown directly on SiO_2). Asterisk: $p < 0.05$ vs thickness = 0 (LSD *post hoc* tests for multiple comparison).

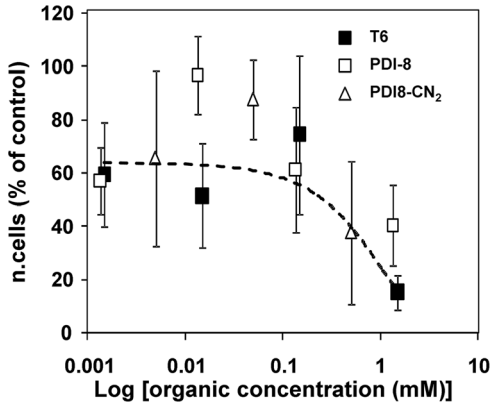

FIG. 5. HeLa cells adhesion on plastic substrates as a function of the T6, PDI-8CN_2 and PDI-8 concentrations in the culture solution. The dashed line is a guide to the eye.

tested whether this occurrence was due to the presence of –CN groups in the PDI-8CN_2 molecular structure, using PDI-8 compound where hydrogen atoms replace the −CN groups. Results showed that the presence of −CN groups did not increase the toxicity of the material, as the decrease of the number of cells as a function of the concentration of PDI-8CN_2 was comparable to that of PDI-8. A two way ANOVA with dose and organic material type as fixed factors revealed that the dose factor significantly changed the number of cells per unit area ($F = 4.79$, $df = 4$; $p = 0.001$), whereas the number of cells per unit area did not significantly change among different organic materials ($F = 2.34$, $df = 2$; $p = 0.10$) without interaction (organic material*dose $F = 1.77$, $df = 8$, $p = 0.09$). We therefore performed a LSD *post hoc* analysis for multiple comparisons for the dose factor only and found that only the highest dose was significantly different.

The toxicity of DMSO vehicle alone was also tested and did not reveal significant changes in the number of cells attached to the plastic substrate after 3 h exposure (cells in basal conditions: 639 ± 130 cells/mm^2; cells with addition of DMSO vehicle: 807 ± 141 cells/mm^2; $p > 0.05$; t-test for non paired data).

Figure 6 confirms that the adhesion of cells on thin organic films has low effect on cell survival after 48 h. Specifically, FDA staining showing living cells (green in Fig. 6(d) online) revealed lower number of cells on PDI-8CN_2 films, in agreement with cell counting data ($F = 4.17$, $p = 0.037$); moreover, the number of cells grown on T6 and SiO_2 surface

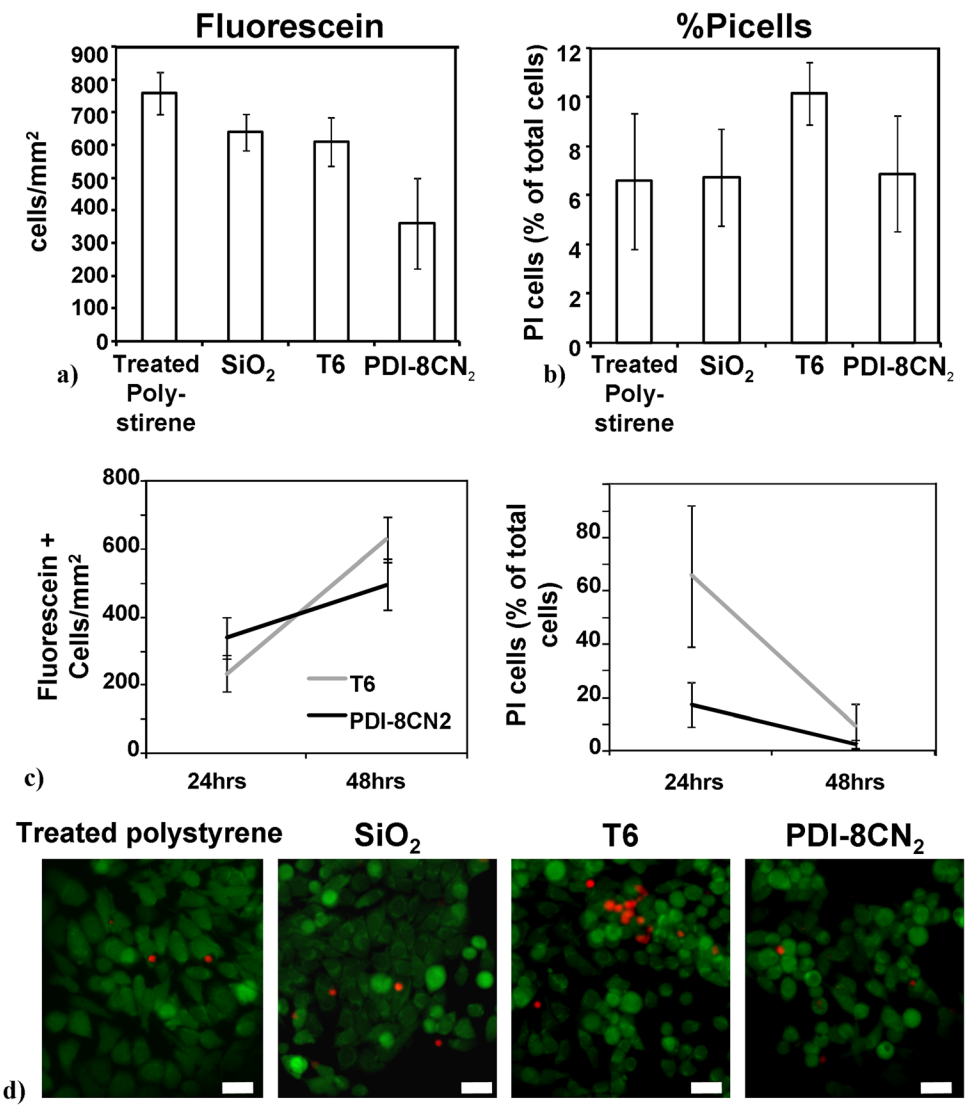

FIG. 6. (Color online) Effect of T6 and PDI-8CN_2 on vitality of HeLa cells. (a) Number of FDA-positive, viable cells per unit area on different substrates. (b) Number of dead cells stained with PI exclusion technique. (c) Number of viable cells (FDA positive) and dead cells (PI positive cells) growing on substrates at 24 h and 48 h on T6 and PDI-8CN_2 substrates. (d) Representative images of HeLa cells grown on different substrates and stained with FDA and PI. Scale bar: 20 μM; asterisk: $p < 0.05$ PDI-8CN_2 vs SiO_2 (LSD *post hoc* tests for multiple comparison).

was comparable, in agreement with previous results, and was smaller than the number of cells grown on plastic substrates, although this result was not significant using multiple comparisons after ANOVA analysis. The difference in the number of living cells among these conditions was not due to a larger number of dying cells, as shown by PI staining (nuclei of dead cells are red in Fig. 6(d) online): in fact, the percent of PI positive cells was similar across different conditions at 48hrs, thus suggesting that the organic materials tested are not toxic at the thickness of 30 nm but the presence of PDI-8CN$_2$ can change the adhesion of cells to the substrates or slow down their division rate. Therefore, we also evaluated the growth of HeLa cells on T6 and PDI-8CN$_2$ substrates at shorter time intervals. Data shown in Fig. 6(c) reveal that seeded cells can grow on both organic materials. Moreover, at 24 h a larger number of dead cells was evident in the preparation with T6 organic material, whereas at 48 h approximately the same proportion of dead cells was evident in T6 and PDI-8CN$_2$ preparations. Since at 24 h the number of cells attached to T6 and PDI-8CN$_2$ substrates is similar, it is plausible that HeLa cells have a slower growth rate on PDI-8CN$_2$. Finally, when considering the morphology of cells grown on PDI-8CN$_2$ they show little amount of cellular processes or spines both for HeLa and F11 cells, in comparison with the other substrates. Since no increased cell death was observed, this is likely to result from a lower ability of the cells to adhere to this substrate. Therefore, the adhesive properties of PDI-8CN$_2$ should be improved as HeLa cells show nonoptimal attachment to this substrate, as evidenced by cellular morphology and the number of adherent cells.

PDI-8CN$_2$ transistor operation in cell culture medium

The achievement of organic transistors suitable to be applied in bio-sensing fields is strictly related to the experimental demonstration of their ability to reliably operate in aqueous solutions and, more specifically, in complex media with large ionic concentration. In the recent past, several studies have been devoted to analyze the OFET operation in de-ionized water and state-of-the-art devices, displaying stable and reproducible behavior on time scales of several hours, have been developed by the group of Bao at the Stanford University.[19,20] Conversely, up to date, the operation of organic transistors in complex ionic media has been poorly investigated and many issues remain to be more deeply addressed. Indeed, only in a very recent paper, the degradation effects induced in the electrical behavior of P3HT transistors by the immersion in ionic liquids were investigated.[21] In particular, the role of the different ionic species was analyzed to shed some light about the most critical contributions affecting the device stability. In our work, a similar goal was pursued in the attempt to define the operation limits of PDI-8CN$_2$ transistors in a strongly ionic liquid environment. Our attention was focused only on PDI-8CN$_2$ active layers, owing to the good perspectives given by perylene diimide compounds for the fabrication of air-stable n-type transistors. For this purpose, a standard transistor configuration, based on SiO$_2$ dielectric barriers and bottom-contact gold source-drain electrodes, was used. T6 transistors on SiO$_2$ substrates were not considered in these experimental tests, mainly because of the poor electrical performances of these devices when measured in ambient conditions. As an ionic liquid, the same type of Dulbecco's modified medium (DMEM) utilized for the culture of F11 cells was considered. First of all, we tested the operation of PDI-8CN$_2$ transistors fabricated on 200 nm thick SiO$_2$ layers, when the active channels were completely covered by the DMEM culture liquid. In air, these devices can normally operate in the gate-source voltage (V_{GS}) range between -50 V and 50 V and with the drain-source voltage (V_{DS}) voltage up to 50 V, displaying stable electrical performances.[22] On the contrary, in the DMEM liquid, the output curves in Fig. 7(a) clarify that the ionic contributions to the overall current start to be dominant for drain-source (V_{DS}) voltages between 1 and 2 V. Furthermore, the leakage current I_{GS} measured between gate and source noticeably increased for negative V_{GS} voltages lower than −5 V (Fig. 7(b)). All these occurrences were experimentally observed for all the investigated devices. This finding confirms the need for low-voltage operation transistors in order to limit the ionic currents and also to prevent the presence of Faradaic contributions coming from the water electrolysis. In good agreement with our results, other reports suggest that V_{DS} values lower than 1 V/2 V must be considered to reduce this last effect.[23]

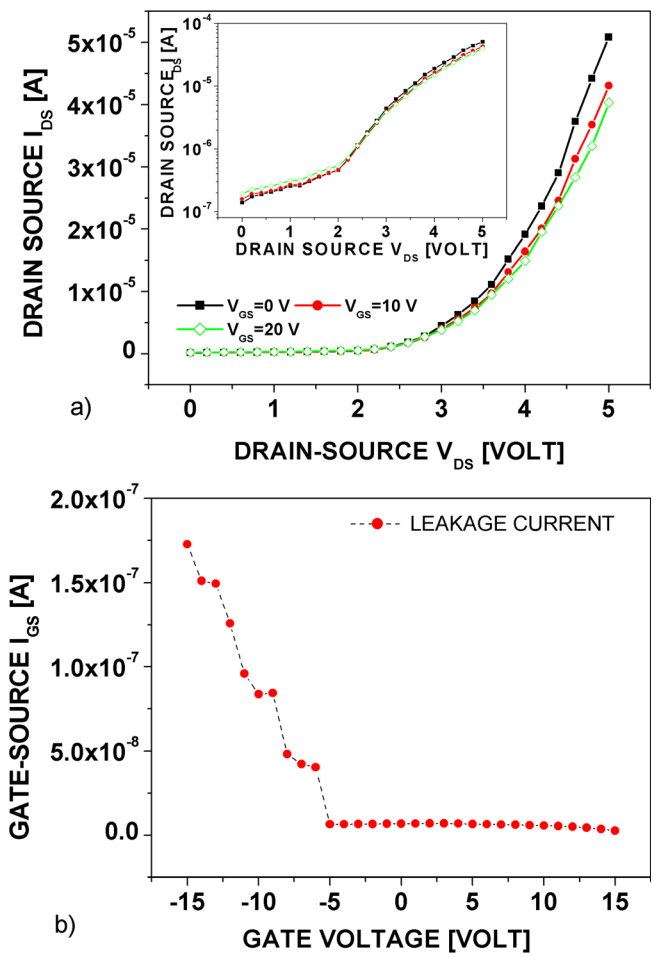

FIG. 7. (Color online) (a) Output curves and (b) leakage current I_{GS} of a PDI-8CN$_2$ transistor, fabricated on 200 nm thick SiO$_2$ layer, operating in the DMEM culture liquid.

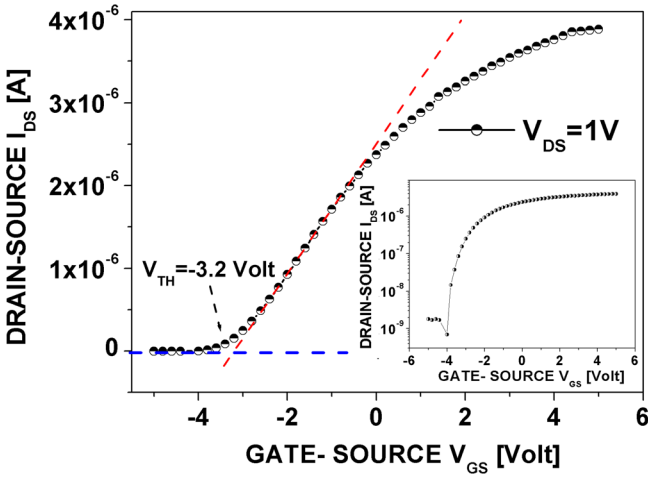

FIG. 8. (Color online) Transfer-curve in linear regime ($V_{DS} = 1$ V) measured in air of a low-voltage operation PDI-8CN_2 transistor.

Starting from the previous results, low-voltage operation transistors have been fabricated by evaporating PDI-8CN_2 films on Si^{++} substrates provided of 35 nm thick SiO_2 layers, while keeping fixed the values of the active channel width ($W = 22$ mm) and length ($L = 40$ μm). Figure 8 reports a typical transfer-curve ($V_{DS} = 1$ V) of a PDI-8CN_2 transistor measured in air. As shown, V_{GS} is swept between -5 V and $+5$V. Similarly to other PDI-8CN_2 transistors fabricated on bare SiO_2 substrates, the threshold voltage is largely negative with values between -3.5 V and -3 V.[9] The onset voltage (V_{on}), defined as the voltage where the drain-source (I_{DS}) current exceeds the lower limit of the experimental set-up (1 nA), is very close to -4 V. Given these values, the fabricated transistors display a normally-on operation in air, with a considerable amount of current even when $V_{GS} = 0$ V. Maximum ON-OFF current ratio is higher than 10^3, while the slope of the transfer-curve (the so-called trans-conductance $g_m = \delta I_{DS}/\delta V_{GS}$) decreases for increasing V_{GS} values in the positive range. This effect should be mainly related to the presence of non-negligible contact resistances (R_C) at drain-source electrodes. For our transistors, the maximum values of the trans-conductance g_m range between 0.2 and 1 μS, being reached in the V_{GS} region between -1 V and 0 V. From the experimental transfer-curves, charge carrier mobility μ values between $2*10^{-3}$ and 10^{-2} $cm^2/V*s$ have been extracted by using the ideal MOSFET equations in linear regime:

$$\mu_{lin} = g_m * \frac{L}{WC_{OX}V_{DS}}, \quad (1)$$

where C_{OX} ($\sim$98 nF/cm^2) is the dielectric barrier capacitance per unit area.

Figure 9(a) presents the time evolution during 4 h of the transfer-curve ($V_{DS} = 1$ V) of a PDI-8CN_2 device, when its active channel was covered with the DMEM culture liquid. In the V_{GS} range between -3 and 5 V, the device showed to be still able to work and the current enhancement driven by the electrostatic modulation always exceeds 1 μA, being much higher than the I_{GS} leakage current (Fig. 9(b)). However, it is also evident an increase with time of the I_{DS} current at any V_{GS}. In particular, after the initial bump that

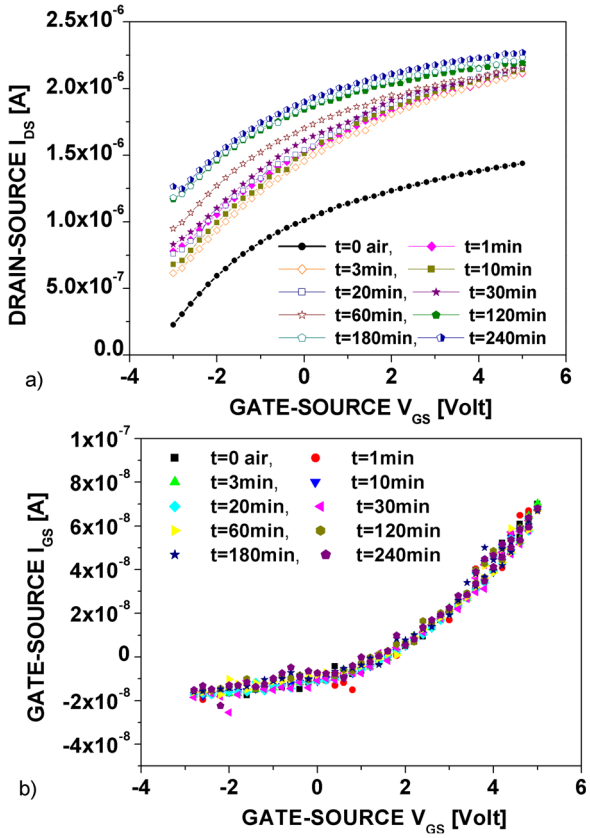

FIG. 9. (Color online) (a) Transfer-curves and (b) the corresponding leakage current curves of a low-voltage operation PDI-8CN_2 transistor operating in the DMEM culture liquid.

can be observed after few seconds, I_{DS} appears to increase faster for negative V_{GS} than for positive ones (Fig. 10(a)). This occurrence suggests that, in this condition, a considerable doping effect coming from the action of the ions in the DMEM solution takes place. The physical nature of this doping and its dynamics are very interesting subjects, deserving further investigation. From the experimental data in Fig. 9(a), by using Eq. (1), the dependence of maximum mobility on the immersion time is extracted and plotted in Fig. 10(b). Significantly, mobility exhibits an increasing behavior in the first 10 min followed by a slightly decreasing trend, which can be described by the first order exponential decay law $\mu(t) = [A*e^{-(t/\tau)} + \mu_f]$. The best fitting curve is obtained with τ and μ_f equal to 63 min and 0.00138 $cm^2/V*s$, respectively. The increasing of the mobility values found few minutes after the transistor immersion resembles the results from Bao group, concerning the electrical behavior of different types of organic transistors in de-ionized water.[2] Considering the overall time evolution, mobility value after 4 h is reduced by only 10% in comparison with the initial one measured in air. After the experiments in DMEM, the surfaces of all tested transistors were observed by an optical microscope. Similarly to other experimental tests reported in literature,[5] this analysis confirmed that the surface of the organic layer suffered evident effects of delamination only in the regions near the source-drain electrodes, where corrosion phenomena[23] appear to compromise the structural stability of the films. This occurrence strongly highlights the need of an adequate

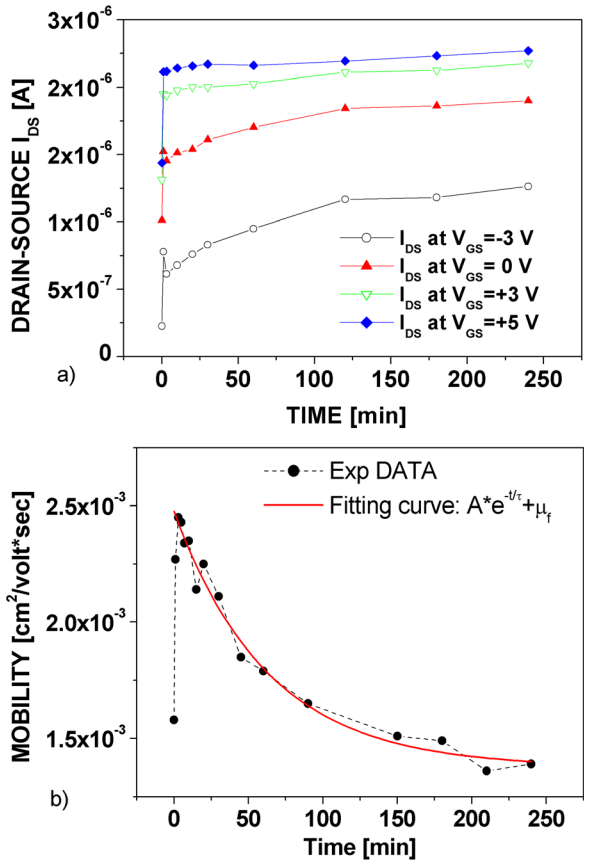

FIG. 10. (Color online) (a) Transistor currents at different V_{GS} and (b) extracted mobility plotted as a function of the immersion time in the DMEM liquid. The solid line is the best fitting curve with $\mu(t) = [A^*e^{-(t/\tau)} + \mu_f]$.

protection of the contact electrodes to further stabilize the electrical response of OFETs operating in ionic liquids.[19]

CONCLUSIONS

Cell culture experiments reported in this work support the conclusion that, even if their adhesive properties should be improved, PDI-8CN_2 films represent an interesting active layer for the fabrication of cell-compatible n-type organic transistors. Indeed, although this material exhibits some degree of toxicity on cells in active replication (HeLa cells), thereby slowing the formation of a confluent monolayer, F11 neuronal cells can attach to this substrate and survive adequately. Very interestingly, F11 cell attachment and survival showed to be dependent on the film thickness, improving when thinner (10-15 nm) films (which are the most appealing for the application in the bio-sensing field) are used. Low-voltage operation PDI-8CN_2 transistors fabricated on 35 nm thick SiO_2 layers display a normally-on mode operation in air, with the maximum of the trans-conductance (g_m) in the gate voltage (V_{GS}) region between -1 and 0 V. Testing these devices in F11 cell culture medium and keeping V_{GS} in the range between -3 V and 5 V and $V_{DS} = 1$V, Faradaic currents related to the large ionic concentrations and water electrolysis are avoided. While the transistor current at any V_{GS} displays a continuously increasing behavior as a function of the immersion time, the trans-conductance and the related field effect mobility obey a non-monotonic law, with a maximum after a few minutes of immersion and a subsequent decreasing evolution. The trans-conductance values after four hours of immersion are, however, comparable to those initially recorded in air. At this stage, the structural and electrical properties of the contact regions near the source-drain electrodes seem to be the most critical in order to improve the operation stability of these devices. Future work will be mostly devoted to design strategies able to address this important issue.

ACKNOWLEDGMENTS

Financial support from the Italian Ministry of Research under PRIN 2008 program "*Investigation of n-type organic materials for electronic applications*" and from the regional projects FARO "*Fabrication of organic transistors for bio-sensing applications*" and MEDIREN "*Innovative methods and devices for the detection of electrophysiology parameters from neuronal cells and tissues*" are gratefully acknowledged.

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