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A Solution for Biocompatibility Problems

Advantages and applications of low-operating voltage organic thin-film transistors.

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THERE IS AN INCREASING NEED in medical diagnosis for reliable, fast, simple, and low-cost biosensing devices. Technological progress has been achieved using multifunctional and multiparameter test platforms, where several (bio)chemical and physical sensors are integrated for the construction of micrototal-analysis systems, lab-on-chip devices, and electronic sense organs [1]. These chip-based technologies or biochips are small sample holders on which biological material is deposited for sample preparation, chemical/biological reactions, and analytical purposes. By shrinking the total reaction volume, the analysis can be carried out more rapidly and at lower cost than with current laboratory bench-scale methods.

Small, portable, disposable, and inexpensive sensors, which can be used as disposables in life-science applications, cover a wide range of sensing applications, i.e., food and environmental monitoring or detection of biological hazardous material. Sensors based on organic semiconductors combine the addressed features. They are flexible and electrically conductive and can be produced in large areas with low cost [2]. In recent years, organic biosensors based on electrical

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(or electronic) transduction mechanisms came into the focus of several groups. A remarkable number of examples can already be found in the literature. Among these transistor-based devices (such as gas, vapor, and fluid sensors) [3]–[7], ion-sensitive field-effect transistors (ISFETs) [8]–[11], sensors used in liquids [12]–[15], and organic electrode materials used in ampero/voltammetry [16]–[20] are in use. Unfortunately, for the applications in life sciences, biocompatibility and biofunctionalization of conducting and semiconducting polymers is often lacking.

Here, we describe the biocompatibility of solution-processable organic semiconducting polymers. Low-operating voltage organic thin-film transistor (OTFT) devices have been fabricated, which can be used as sensors in electrolytes. The devices are based on regioregular poly(3-hexylthiophene) (P3HT), being both a reasonably conductive and optically active polymer. To overcome biocompatibility problems, protein-based coatings and oxygen-plasma treatments have been adopted to enable growth of adherent living cells on those modified surfaces. With the demonstration of biocompatible semiconducting polymeric layers, a hurdle for the realizations of low-cost and mass-produced sensors in life science has been overcome. This opens new possibilities and applications of biological sensing with organic electronic devices.

ORGANIC THIN-FILM TRANSISTORS

Bottom-gate OTFTs based on solution-processable P3HT (regioregular, electronic grade, 99.995% trace metals basis, number-average molecular weight M_n 15,000–45,000) were fabricated on highly p-type doped Si wafers, on which an 80- or 45-nm-thick SiO_2 layer was thermally grown in a dry oxidation process at 1,000 °C. A multifinger electrode structure consisting of a 2-nm-thick Ti layer (as adhesion promoter) followed by a 45-nm Au layer was evaporated on top of the SiO_2 and patterned by lift-off procedures in an optical lithographic step. The channel length, i.e., the spacing between source and drain contacts of the interdigitated structure, was chosen between 10 and 50 μm.

Regioregular P3HT was used as a semiconductor material for the channel of our p-type OTFT and was deposited on top of the sample from a solution of 1 wt% concentration in toluene or chloroform. The P3HT solution is prepared with 0.06 g of P3HT in 6 g of chloroform or toluene (1 wt%) in a glass bottle with a magnet inside. The P3HT solution is put in an ultrasonic bath for 15 min so as to dissolve the P3HT material completely into the solvent. Both spin-coating [21] and spray-coating [22] deposition techniques were successfully applied. The polymer and the dielectric surface was not modified. All the electrical characteristics of the OTFTs were measured using a semiconductor characterization system (Keithley 4200). The analysis has been carried out at room temperature under ambient conditions. Because of the interdigited electrode structure used as electric contacts, the channel has a large cross-section area (W/L). A detailed description of the performance of the OTFTs along with theoretical analysis has recently been reported [21].

A schematic cross section of the transistor structure with optical images of the samples as well as output characteristics of a representative device with 20-μm channel length and a W/L = 13,350 are shown in Figure 1. As the surface of the silicon dioxide was not separately treated, nearly all transistors showed a positive threshold voltage of about +5 V, thus displaying a clearly conducting channel already at a zero gate voltage, as can be seen in Figure 1. The field-effect mobility extracted from the transfer characteristics of the transistors was in the order of 10^{-4} $cm^2/V \cdot s$, which was consistent with previous findings and experiments [23].

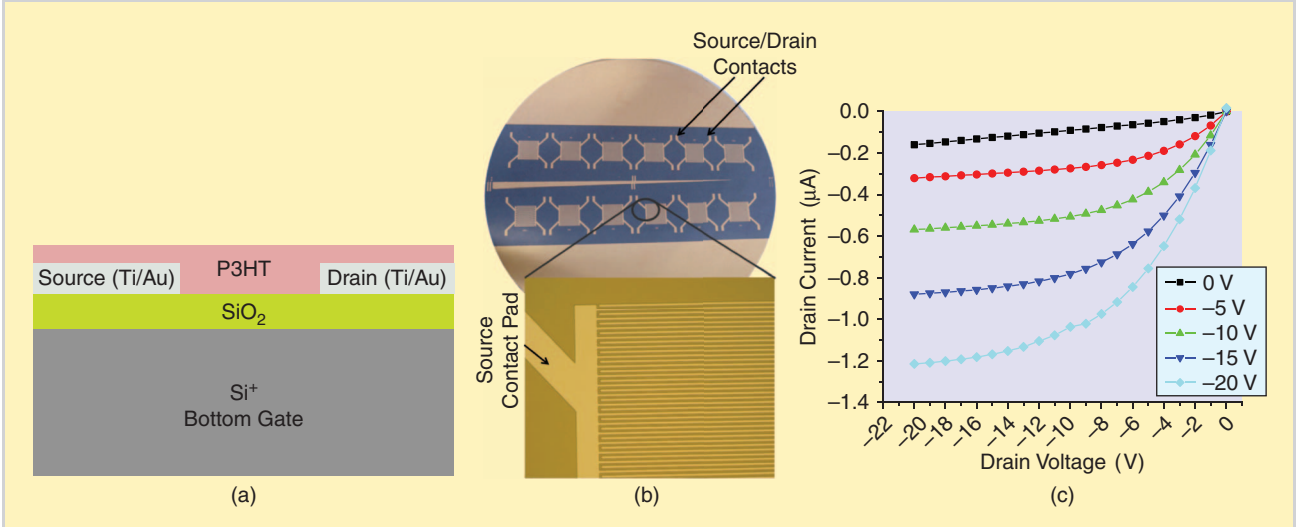

FIGURE 1 (a) Cross-section schematic structure of the OTFT. (b) Optical image of the interdigitated source/drain layout used for the OTFT. (c) Output characteristics of an OTFT with P3HT spun out of toluene with a channel length of 20 μm and W/L = 13,350 on 80-nm silicon dioxide. The OTFTs have been fabricated on 2-in boron-doped silicon wafers, which act as a gate.

BIOCOMPATIBILITY AND FUNCTIONALIZATION

Thiophene-derivates, such as P3HT, represent promising organic materials. Normally, they are characterized by a good solubility, ease of processing, and good environmental and thermal stability. Therefore, P3HT biocompatibility and biofunctionalization have been investigated. P3HT solutions in toluene or chloroform were deposited and cured at temperatures higher than the boiling point of the used solvent, yielding nontoxic, solvent-free uniform layers. For biocompatibility and cell adhesion, and to evaluate the effect of surface treatments and protein-based coatings, cell-growth studies and adhesion experiments on the modified P3HT thin-film layers have been carried out with L929 mouse fibroblasts. L929 mouse fibroblasts derived from normal subcutaneous areolar and adipose tissue were grown in RPMI cell culture media supplemented with 10% fetal calf serum (Figure 2). The spindle shape states the adherent growth of the cells on the underlying surface. Thereby, the surface characteristics have a significant impact on the adhesion, differentiation, and proliferation of surrounding cells.

For biocompatibility studies, glass substrates were coated with thin P3HT layers, by spinning and casting the polymer under nitrogen atmosphere. The latter method was applied to obtain films in the range of 100 nm to 10 μm thickness. These hydrophobic coatings have not been changed further. The details on the biocompatibility and biofunctionalization studies of P3HT are published separately, along with all relevant experimental details [24]. Here, we briefly summarize the methodological steps. The first step in the test series for biocompatibility of the polymer was to plate cells on the sensor surface without any further treatment besides the solvent removal under nitrogen atmosphere. The cell-growth experiments were carried out on P3HT-layers as follows: to sterilize the chips, they were rinsed with 70% ethanol and placed under ultraviolet (UV)-C radiation for 20 min. Subsequently, a well-defined concentration of cells was seeded onto the chips and left to grow in an incubator (Forma Scientific CO_2 water-jacketed incubator) at 37 °C and 5% CO_2. In these initial experiments, it could be shown that, even after 24 h of incubation, the cells did not adhere to the surface and were still in their nonadherent round shape. The formation of a healthy morphology/cytoskeleton and a stable sticking of cells represent an obligatory precondition for all further studies of chemophysical interaction between organisms and electroactive polymers/materials. Thereby, surface characteristics, such as charge density and hydrophobicity, play a key role in protein adsorption and cell–substrate interactions. It has been shown that both cell–surface interactions and cellular functions (e.g., DNA synthesis) on thin polypyrrole films can be controlled by either changing the oxidation state of the polymer or by changing the wettability (hydrophobicity) of the polymer film using appropriate dopants [25]. Since the cells poorly adhere to the hydrophobic surface of the chip, functionalizing coating solutions, such as fibronectin, poly-L-lysine, and collagen, were deposited onto the chips to improve the adhesion of the cells.

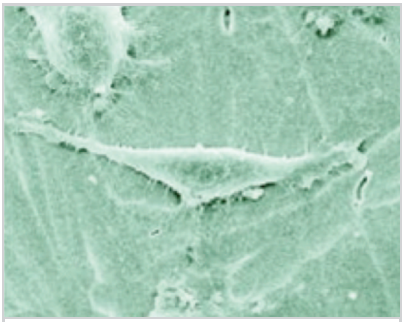

FIGURE 2 Morphology (200× magnification) of the mouse connective tissue fibroblasts cells (L929) on sterilized glass substrates, established from the normal subcutaneous areolar and adipose tissue of a C3H/A mouse used as target in TNF detection assays.

Optical microscopic (Zeiss LSM 510) images were used to quantify the treatment effects on cell proliferation (Figure 3). The protein-based coatings were applied under sterile conditions onto the chip surface and incubated for 30 min at 37 °C. After incubation, the chip was rinsed with phosphate-buffered saline before the cell seeding. Unlike the untreated biosensor, the growth increased significantly. The L929 cells attach and differentiate equally well on fibronectin and on collagen protein-based coatings and more poorly on poly-L-lysine [24]. It is worth mentioning that the cell-growth experiments have been performed many times, and a very good reproducibility of the results has been found. These experiments have been performed on plasma-oxidized surfaces, showing how a well-controlled wettability of the surface could allow adherent cell growth. The surface energy of the organic polymer can be considerably modified as shown by a decrease in the water contact angle (Figure 4). Without any treatment, the P3HT surface is very hydrophobic [contact angle close to 90°, Figure 4(a)].

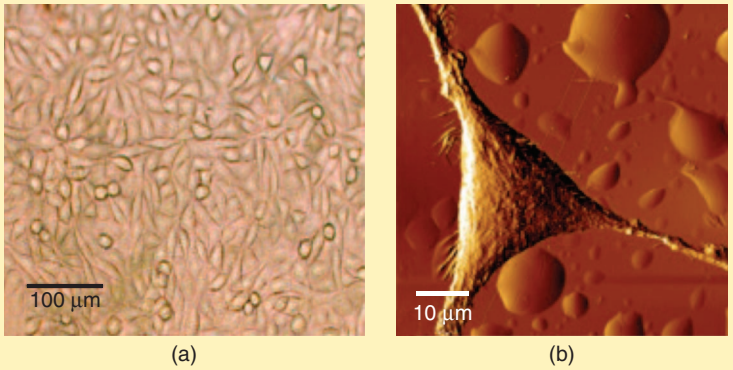

FIGURE 3 (a) Optical microscopic image of mouse fibroblasts confluently grown on collagen-functionalized P3HT polymeric surface. (b) High-resolution AFM image of a single-cell fixed on the polymeric layer.

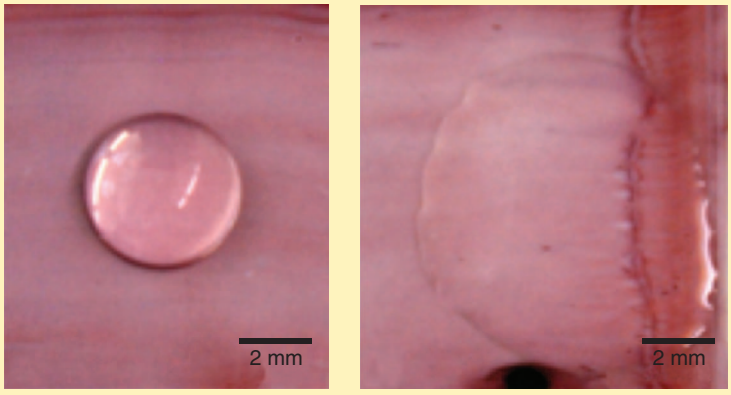

FIGURE 4 Optical images of P3HT-coated glass substrates with deionized water droplets on top before and after a controlled plasma oxidation. (a) Because of the hydrophobicity of the surface, the droplet flow is hampered (contact angle close to 90°). (b) The wettability of the surface has been changed by the oxidation process.

After treating the polymeric surface with oxygen plasma for 1 min, the water contact angle decreases dramatically [Figure 4(b)]. This flexibility of engineering semiconducting polymer layers offers the possibility of patterning a surface with adhesive and nonadhesive areas. As can be seen in Figure 4, a well-controlled plasma oxidation provides varied surface characteristics, on which liquid media (water droplets) can be spotted.

After performing these treatments on a transistor structure, the electrical characteristics of the device did not change significantly (data not shown), and the transistor behavior shown in Figure 1(c) could be reproduced. Finally, to observe the effects of P3HT on the typical morphology of this cell type, confocal microscopy (Figure 5) and atomic force microscopy (AFM) imaging studies were carried out on cultured L929 fibroblasts after 24 h. High-resolution AFM-images of cells fixed with glutaraldehyde on a collagen pretreated P3HT chip are shown in Figure 3(b).

FUTURE WORK AND PERSPECTIVE

For the first time, we have successfully performed the cell-growth experiments on biofunctionalized P3HT thin films, which can easily be adapted to other organic layers. OTFTs with low-driving voltages for biomedical applications using biocompatible P3HT polymeric layers have also been fabricated. The ability and flexibility at the level of controlling polymer surface properties provide tools to engineer materials that yield predictable and desirable cell–surface interactions. A substantial hurdle for the realization of low-cost and mass-produced sensors in life science has been overcome. Therefore, organic semiconducting polymers, such as P3HT, will become interesting candidates for a range of medical applications, by virtue of inherent semiconducting electrical properties. The electrical and conducting properties of the polymer are not affected by the treatments performed, hence allowing an easy adaptation to other organic semiconducting polymer layers.

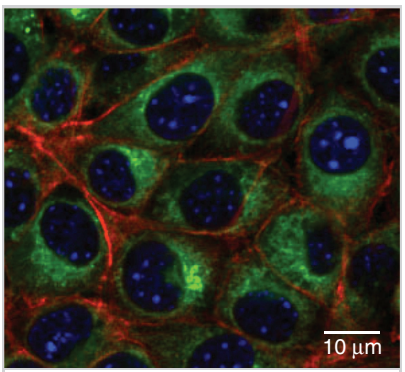

FIGURE 5 Confocal microscope image of fibroblasts grown and stained on the polymer surface. The cell membrane was labeled with DiO; after fixation and permeabilization, the F-Actin was stained with Rhodamine Phalloidin, and the nuclei were counterstained with Hoechst 33342.

For future applications, AFM-based elasticity measurements should determine the quality of cell adhesion. The long-term target is a sensor structure that is able to convert cell–cell communication processes into electronic signals. The work presented here marks the first step in this direction. The next step will be the investigation of the cell–polymer interaction under the influence of electrical voltages. For the development of impedance sensors and amperometric oxygen sensors, electric lines of organic materials will be needed. For environmental applications (e.g., pH measurements), organic ISFETs based on the presented structures have been already realized [26]. In this case, in addition to thin insulating layers, semiconducting organic materials will be provided.

Organic sensors can be fabricated on flexible foils and hence are capable of bending and stretching. This is a major advantage of organic sensors compared with silicon semiconductor-based sensors. Furthermore, they are much simpler and thus, also for small and medium volumes, significantly cheaper to produce. This, for instance, can be of particular interest for skin or body implantable sensors, which must reliably work under constant movement. Furthermore, organic structures can be applied to large areas; thus, the necessary number of cells statistically contributing to the sensor can be answered here in a simple manner by providing corresponding big surfaces. At the same time, a printing production line (also in a relatively simple and inexpensive form) can be adjusted to single situations. Parallel to this, an important issue would be to develop special contacting and encapsulation techniques.

Based on the realization of biomedical sensor structures made from organic materials, a well-suited characterization set-up, accessing the electrical properties of these sensors, should also be designed at a circuit level. The implementation of these circuits with already existing life-support systems will lead to new test systems for various monitoring applications in life sciences.

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