Society For Biomaterials

Printing inks of electroactive polymer PEDOT:PSS: The study of biocompatibility, stability, and electrical properties

Stanislav Stříteský,[1] Aneta Marková,[1] Jan Víteček,[2,3] Eva Šafaříková,[2,4] Michal Hrabal,[1] Lubomír Kubáč,[5] Lukáš Kubala ,[2,3] Martin Weiter,[1] Martin Vala [1]

[1]Materials Research Centre, Faculty of Chemistry, Brno University of Technology, Purkyňova 118, Brno 612 00, Czech Republic
[2]Institute of Biophysics of the Czech Academy of Sciences, Královopolská 135, Brno 612 65, Czech Republic
[3]International Clinical Research Center-Center of Biomolecular and Cell Engineering, St. Anne's University Hospital Brno, Pekařská 53, Brno 656 91, Czech Republic
[4]Department of Experimental Biology, Faculty of Science, Masaryk University, University Campus Bohunice, Kamenice 5, Brno 625 00, Czech Republic
[5]Centrum Organické Chemie, Rybitví 296, Rybitví 533 54, Czech Republic

Received 22 September 2017; revised 5 December 2017; accepted 15 December 2017
Published online 17 January 2018 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.a.36314

Abstract: Biocompatibility tests and a study of the electrical properties of thin films prepared from six electroactive polymer ink formulations based on poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) were performed. The aim was to find a suitable formulation of PEDOT:PSS and conditions for preparing thin films in order to construct printed bioelectronic devices for biomedical applications. The stability and electrical properties of such films were tested on organic electrochemical transistor (OECT)-based sensor platforms and their biocompatibility was evaluated in assays with 3T3 fibroblasts and murine cardiomyocytes. It was found that the thin films prepared from inks without an additive or any thin film post-treatment provide limited conductivity and stability for use in biomedical applications. These properties were greatly improved by using ethylene glycol and thermal annealing. Addition or post-treatment by ethylene glycol in combination with thermal annealing provided thin films with electrical resistance and a stability sufficient to be used in sensing of animal cell physiology. These films coated with collagen IV showed good biocompatibility in the assay with 3T3 fibroblasts when compared to standard cell culture plastics. Selected films were then used in assays with murine cardiomyocytes. We observed that these cells were able to attach to the PEDOT:PSS films and form an active sensor element. Spontaneously beating clusters were formed, indicating a good physiological status for the cardiomyocyte cells. These results open the door to construction of cheap printed electronic devices for biointerfacing in biomedical applications. © 2018 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 106A: 1121–1128, 2018.

Key Words: conducting polymer, biointerface, biomedical applications, biosensor, biocompatibility

How to cite this article: Stříteský S, Marková A, Víteček J, Šafaříková E, Hrabal M, Kubáč L, Kubala L, Weiter M, Vala M. 2018. Printing inks of electroactive polymer PEDOT:PSS: The study of biocompatibility, stability, and electrical properties. J Biomed Mater Res Part A 2018:106A:1121–1128.

INTRODUCTION

Poly(3,4-ethylenedioxythiophene) (PEDOT) has become one of the most widely-used organic semiconductors today. A mixture of it with poly(styrene sulfonate), leading to macromolecular salt (PEDOT:PSS), has been used as a charge carrier injection or extraction barrier reducing material and hole-conducting and/or charge (electron) blocking layer in various organic electronic devices such as (printed) organic solar cells (OPV) and organic light emitting devices (OLED).[1–3] Beside these applications, a new and particularly interesting field has recently arisen. It has been shown that PEDOT:PSS and its derivatives can be used as a part of a sensing device for detection of a myriad of chemical compounds, including biologically relevant ones such as dopamine, ascorbic acid, and so forth.[4]

However, PEDOT:PSS is today probably mostly studied for its ability to conduct both electrons (holes) and ions. This mixed electronic–ionic conductivity allows creating devices with new modes of operation such as batteries and supercapacitors, electromechanical actuators, electrochromic windows, solid electrolyte capacitors and, most importantly (with respect to this work), organic electrochemical transistors (OECTs).[5] PEDOT:PSS-based electronic devices can act both as efficient transducers of living cells' activity

Additional Supporting Information may be found in the online version of this article.
Correspondence to: Martin Vala; e-mail: vala@fch.vut.cz
Contract grant sponsor: Grantová Agentura Českę Republiky; contract grant numbers: 13-29358S, 17-24707S
Contract grant sponsor: Ministerstvo Školství, Mládeže a Tělovýchovy; contract grant number: LO1211

TABLE I. The Studied Materials: Notation, Commercial Name and Description from the Supplier

Notation	Commercial name	Description
Ink A	Heraeus Clevios^{TM} P VP AI 4083	Standard dispersion for hole injection layers (OPV, OLED), pH acidic
Ink B	Heraeus Clevios^{TM} P JET HC V2	Ink-jet formulation, high conductive version of Clevios^{TM} P, acidic
Ink C	Sigma Aldrich 739 316	Optimized for ink-jet, contains 1–5% ethanol, 5–10% diethylene glycol, concentration 0.8% in H_2O, acidic (pH 1.5–2.5)
Ink D	Sigma Aldrich 483 095	Conductive grade, contains PEDOT 0.5 wt %, PSS 0.8 wt %, concentration 1.3 wt % in H_2O
Ink E	PEDOT/PSS-AS	Contains PEDOT 0.4 wt %, PSS 0.6 wt %, concentration 1 wt % in H_2O, no additives
Ink F	Sigma Aldrich 560 596	Hole injection layer in OLED, low conductivity grade, contains PEDOT 0.14%, PSS 2.6%, concentration 2.8 wt %, acidic (pH 1.2–1.8)

(sensors) or produce stimuli by ion injection (ion pump) for living tissue engineering.[6] Due to the favorable mechanical compatibility of PEDOT:PSS with living tissues (this remains true for most of organic semiconductors, not only PEDOT:PSS), this material is becoming a cornerstone of applications where functional electrical devices interact with living tissue, such as biosensing, neural interfacing, implant or prosthesis engineering, drug delivery applications, and so forth.[7]

To fabricate such devices, several technologies can currently be used. Among them, mainly material printing is believed to be the most suitable for high-throughput manufacturing. Printing can bring substantial cost reductions compared to other technologies such as lithography and still provide adequate lateral resolution and repeatability[8–10] for a variety of applications. However, to fabricate devices for biointerfacing by any of the printing techniques, several requirements for the printing inks (formulations) have to be met. These concern mainly viscosity, surface tension, processing conditions, ink aggregation, and so forth. A common strategy to prepare the printing formulations is to adjust the inks' properties using additives. As a result, the inks contain several chemical compounds (each manufacturer uses different ones, which usually remain unknown) that can affect the resulting electrical properties but mainly the biocompatibility and stability of the devices in a biological environment.

In the context of working with living cells that require a solid support (the vast majority of animal cells), biocompatibility is defined in terms of the absence of cytotoxicity and the ability to provide support for cell adhesion and spreading (i.e., the establishment of contact with the surface). This results in cells with correct morphology and sustained viability. Such properties are largely determined by the release of potentially cytotoxic compounds[11,12] as well as the ability of the surface to provide loci which allow cells to attach and spread.[13,14]

In order to determine the suitability of commercially available PEDOT:PSS print inks for the construction of bioelectronic devices (namely OECT), biocompatibility tests of five commercially available PEDOT:PSS formulations were performed. One formulation prepared in our labs was used as a reference. To improve the biocompatibility and electrical conductivity, additional treatments of the prepared thin films were used. Thermal annealing and treatment with higher glycols (an ethylene glycol in our case) were tested. The tests were done for thin films deposited on an electrode system of an OECT device to provide higher reliability of the obtained results. The stabilized films were then successfully tested for biocompatibility using 3T3 fibroblasts and murine cardiomyocytes.

EXPERIMENTAL

Materials

Five commercially available PEDOT:PSS inks (Ink A–D and Ink F) in the form of water suspension were used, see Table I. Ink E was prepared in our laboratory and since we know its exact composition, it was used as a reference material here. The PEDOT:PSS was prepared following the procedure reported in Ref. 15. The polystyrenesulfonic acid (PSS) had a molecular weight (Mw) of 1,000,000.

Samples preparation and characterization

To prevent possible errors arising from different properties of the tested films prepared by different printing techniques, we used spin-coating to create the films with a comparable film morphology and thickness for all ink formulations. Prior to the spin coating, the substrates were cleaned, rinsed in isopropanol and sonicated for 10 min, The PEDOT:PSS formulations were filtered using 0.45 μm syringe filters before the spin-coating to avoid deposition of large aggregates. The films were spin-coated at 3000 rpm. The films' thickness was measured by means of a mechanical profilometer (Bruker DEKTAK). The thicknesses were in the range of (30 ± 5) nm. Thermal annealing of the films was performed on a hot-plate at 150°C for 15 min in ambient atmosphere. Whenever ethylene glycol treatment is referred to, a drop of ethylene glycol was placed on the film for 15 min and then washed with deionized water and dried with dry air.

The films' stability in the aqueous environment was monitored with a UV-Vis spectrophotometer Varian Cary 50.

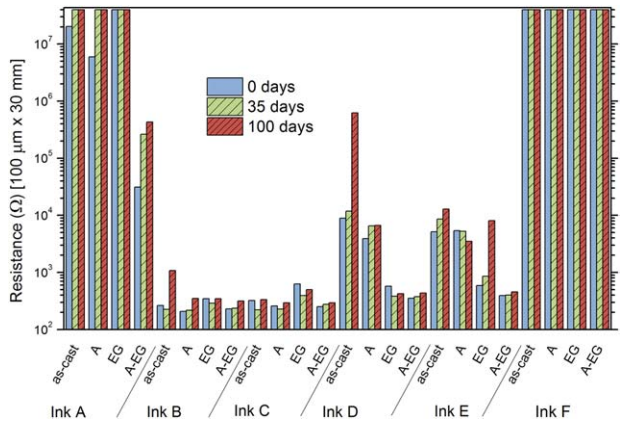

FIGURE 1. Electrical resistances of PEDOT:PSS thin films prepared from studied ink formulations using various treatments. The films were stored in the dark at ambient atmosphere and temperature.

The prepared thin films were immersed in deionized water and absorption was measured as a function of time. The measured absorption at 226 nm was compared against the calibration curve and a relative percentage (V/V) of the dissolved material was evaluated.

Electrical properties of the PEDOT:PSS films were measured using a Keithley 2100. The samples were deposited on glass substrates with interdigitated ITO electrodes of differing channel width (50, 75, 100, 150, and 200 µm) and constant channel length (30 mm) (Ossila Ltd.).

Biocompatibility assay

Thin films of PEDOT:PSS on a 15 mm glass circle (Cover Glass No. 1) were sterilized by immersion into 70% (V/V) ethanol for at least 5 min and washed in physiological buffered saline (PBS 137 m*M* NaCl, 2.7 m*M* KCl, 10 m*M* Na_2HPO_4, 1.8 m*M* KH_2PO_4; pH 7.4) three times for at least 2 min. Biocompatibility was determined by means of 3T3 fibroblasts (ATCC CRL-1658). These were routinely grown in Dulbecco modified Eagle medium, high glucose supplemented with 10% fetal calf serum, 100 U/mL penicillin, 0.1 mg/mL streptomycin (all from Gibco-Invitrogen) as described previously.[16] Inoculum for the biocompatibility assay was set to 15,000 per 1 cm^2. Cells were grown for 48 h.

To determine cell viability MTT or ATP assay were employed. A stock solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-difenyl-2H-tetrazolium-bromide (MTT, Sigma-Aldrich, cat. No 13,503-8) at 2.5 mg/mL was diluted 10 times to the cell culture. Incubation was carried out at 37°C in 5% CO_2 for 4 h. The medium was removed and cells were extracted with 300 µL of 10% triton X100 in 0.01 *M* HCl per sample on a shaker for 15 min. The extract was clarified by centrifugation (5000g, 5 min) and the absorbance was read at 570 nm.

The intracellular adenosinetriphosphate (ATP) assay was carried out using an ATP assay kit (BioVision, K355-100) as per the instructions of the kit manufacturer.

Cardiomyocytes and beating analysis

Cardiomyocytes were differentiated from mouse HG8 line of embryonic stem cells (ATTC) and purified according to Radaszkiewicz et al. 2016.[17] Cardiomyocytes were inoculated into the sensor and beating was quantified by means of video analysis[17] after 24 h.

RESULTS AND DISCUSSION

The PEDOT:PSS polymer films suitable for any of the biomedical applications as an electroactive material should have high electrical conductivity. For example, in the case of devices based on OECT, the higher the conductivity, the higher the resulting transconductance. The transconductance reflects the OECT amplification of the signal that can be obtained[18] and hence the sensitivity of the device. In the case of PEDOT:PSS, the conductivity is primarily dependent on the ratio of the two components. The higher the PSS content, the lower the conductivity. This can also be seen from our results (see Fig. 1) where several orders of magnitude higher resistances were measured for Ink A and Ink F than for the rest of the formulations in the test. While the PEDOT:PSS ratio used in Ink A is not known (the supplier does not provide it), Ink F can be directly compared to Ink D with significantly lower PSS concentration (2.6% vs. 0.8%) and thus with higher conductivity (see Table I).

The other factors that influence the PEDOT:PSS films' conductivity are mainly related to the polymer chains' conformation and packing. This is greatly influenced by the preparation method and conditions during the film formation. A common strategy is to use additives that regulate the solvent evaporation. The additives can be applied prior to the deposition (additives are part of the ink formulation) or can be applied afterwards, upon the prepared thin film. Another condition that has been reported that causes structural changes of the PEDOT and PSS polymer chains is thermal annealing of the prepared films.[3,19]

In this study, we used these additional film-improving techniques in order to find the best conditions for utilization of the commercial PEDOT:PSS inks for the preparation of electroactive thin films for a sensor to detect the activity of living cells for biomedical applications. The thermal treatment will be referred here as "A" and exposure to ethylene glycol will be referred as "EG."

Electrical resistance of the thin films

First, the electrical resistance of the studied materials was compared. Figure 1 shows the measured resistance of the thin films prepared from the studied inks immediately after the preparation and after 35 and 100 days of storage in the dark at room temperature. The resistance was measured for (i) spin-coated films only (as-cast), (ii) thermally annealed films (A), (iii) ethylene glycol treated films (EG), and (iv) both thermal annealed and ethylene glycol-treated films (A-EG). It can be seen that the inks that have not been optimized for high-conductivity applications (Ink A and Ink F) have substantially higher resistance than the other inks in the test. Although the additional procedures to modify film

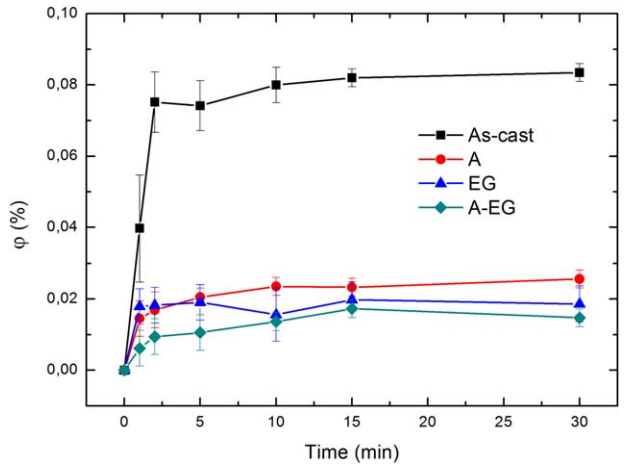

FIGURE 2. Leaching from thin films prepared from Ink B into deionized water. Leaching was evaluated as a volume percentage of the material leached to solvent above the film. ■ as cast films, • annealed, ▲ ethylene glycol treated and ◆ combination of ethylene glycol and annealing.

properties partially lowered the resistance, these inks are not suitable for low-electrical resistance bioelectronic applications (e.g., OECT) and were not further analyzed.

The observed influence of immersing the films into ethylene glycol can be seen from the results obtained for Ink D and the reference Ink E. In both cases the ethylene glycol treatment led to a substantial reduction of the initial films' electrical resistance. On the other hand, Ink C that already contained ethylene glycol shows neither reduction nor increase of the electrical resistance. Ink B for which the supplier does not provide the exact composition showed similar results, suggesting that ethylene glycol (or another additive) is also already included in the ink formulation. We can conclude that it does not matter how the ethylene glycol is applied. The resulting electrical resistances for as-cast films of Ink B and Ink C (both have ethylene glycol added already in the solution) are comparable to the resistances of the Ink D and Ink E after the annealing and ethylene glycol treatment. We can also say that the additional treatment (neither annealing nor ethylene glycol) does not help in further reduction of electrical resistance once the ethylene glycol is included in the ink formulation.

Comparison of the results for freshly prepared and stored films (shelf-life stability testing) does not show a clear trend, see Fig. 1. In some cases (Ink B, Ink D, and Ink E), we observed an increase of the electrical resistance over time for the as-cast films. For Ink D and Ink E and partially for Ink C, we can see that this unwanted aging can be solved by annealing of the films. The films that were treated with ethylene glycol were stable during the testing period and additional annealing did not reduce the obtained resistance fluctuation. The only exception was the reference Ink E where additional annealing of the ethylene glycol-treated films was necessary to stabilize the films for longer term storage. As a very well prepared ink formulation Ink C can be considered. This formulation provides stable films already for as-cast samples.

We can conclude that the thin films prepared from inks without any additive will not provide sufficient performance (electrical conductivity) and stability for use in bioelectronic applications. This can be solved by the treatment used in this work that provides thin films with sufficiently low electrical resistance and its stability over time. In our case, when we used the electrode platforms for OECT with 100 μm channel length and 30 mm channel width, the resistivity has to be below 1 kΩ to provide a reasonably measurable electrical current (for $V_{SD} = 0.5$ V and transconducance 1 mS the $I_{SD} = 0.5$ mA).[16,20] This range was met by Ink B and Ink C without additional treatment and Ink D and Ink E with treatment.

Stability of the thin films in H_2O

Electrical stability and performance in dry conditions (shelf-life) is a necessary prerequisite for bioelectrical sensors, but stability in a cell cultivation medium is even more important. PEDOT:PSS is hydrophilic and thin films immersed into water quickly swell and can partially dissolve. As a result, such a layer undergoes changes to its mechanical and electrical properties and a PEDOT:PSS-based sensor can release compounds that are not naturally occurring in living tissues or culture media. These compounds are potentially harmful and therefore the chemical stability of the PEDOT:PSS surfaces in the physiological aqueous environment can be regarded as a prerequisite for its biocompatibility. Therefore, a simple method to determine PEDOT:PSS thin film dissolution by means of UV-Vis spectrophotometry of the solution above the films was adopted.

To quantify the dissolved material, we compared the observed UV-Vis signal at 226 nm with a calibration curve and calculated the volume percentage of the leached material. Figure 2 shows a typical observed response and its evolution from thin film prepared from Ink B. We can see that the main increase of the signal is within the first few minutes. After this rapid change, the films slowly stabilize. It is also apparent that films that were thermally annealed (A and A-AG) show slower initial leaching. Knowledge of

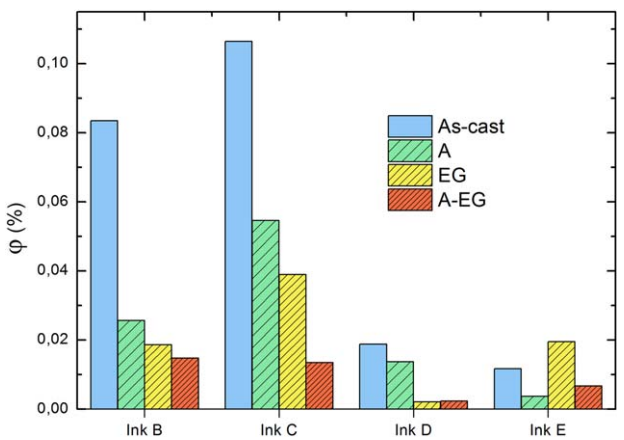

FIGURE 3. Fraction of leached material after30 min exposure to water from thin films prepared by different methods. A, thermal annealing; EG, ethylene glycol treated; A-EG, annealed.

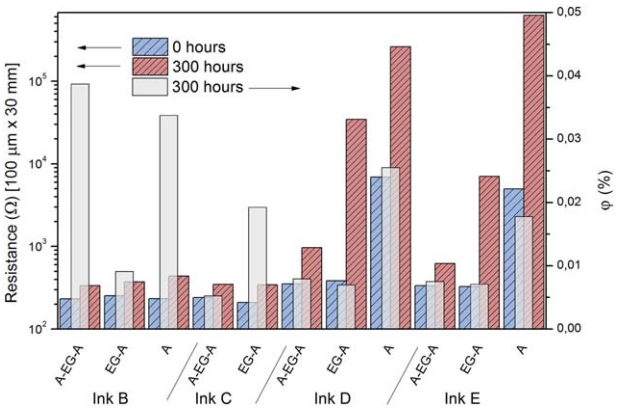

FIGURE 4. Electrical resistance (left axis) and material leaching from the thin films (right axis) prepared by different methods after 300 h exposed to deionized water.

the process dynamics is important for further manipulation of the films before the cells are seeded onto the material. It is common in laboratory practice that prior to cell cultivation, the films are sterilized (e.g., 70% ethanol and three times subsequent washing with PBS) and coated with proteins (application of coating solution for tens of minutes) to increase cell growth and viability.[16] These procedures take from several minutes up to about 1 h. Our data showed that PEDOT:PSS leachables equilibrate with a solution in <2 min (Fig. 2). Hence, preparation of PEDOT:PSS for cell cultures ensures removal of leachables prior to the contact with cells.

The fact that substances from thin films are leached shows that the layer is not stable enough from a long-term point of view and changes in electrical properties can also be expected. Therefore, additional treatments to stabilize the films were performed. The influence of the treatments is shown in Fig. 3. The figure shows the amount of released material after 30 min of exposure to water. We can see that annealing was always beneficial to the tested ink formulations. Ethylene glycol treatment also improved films' stability for Ink B, C, and D but an increase of the released material was observed for Ink E. The combination of the two treatments had a further positive influence in Ink B and Ink C. We can thus conclude that subsequent treatment of the thin films by each of the methods used here or by a combination of them brings substantially improved film stability against water and potential cultivation media. Interestingly, this remains true also for inks with ethylene glycol already included in the ink formulation.

The same test was repeated for thin films stored in the dark at ambient conditions for two months. We did not observe any subsequent differences in the results (data not shown). This means that once the films are stabilized by proper treatment, further storage does not influence the films' stability.

The duration of contact of the sensor with the biological material is highly variable depending on the specific application. In the case of animal cells it can cover a period of up to several days.[21] Thus, to develop a conductive polymer film suitable for working with most animal cell types, the stability of PEDOT:PSS films was tested over a period of 300 h. The films' stability was monitored in the same manner as described previously. Prior to the resistance measurement after the 300 h test, the samples were dried with dry air. The obtained data are displayed in Fig. 4.

Films without any post treatment and films treated with ethylene glycol showed limited stability as they were completely released after 300 h. We observed that thermal annealing is always necessary in order to obtain films stable for several days. However, only thermal annealing alone is not always sufficient. A combination of thermal annealing after the ethylene glycol treatment was always beneficial and improved the films' electrical resistance and stability. The best average results were obtained for Ink C and a combination of annealing–ethylene glycol–annealing.

In Fig. 4, we can again see the positive effect of ethylene glycol treatment on electrical resistance for inks without ethylene glycol in the ink formulation (Ink D and Ink E) at the beginning of the test. However, the long-term stability of thin films prepared from these inks was much worse than for films from Ink B and Ink C that already contain ethylene glycol in the ink formulations. This worse stability can be improved by a second annealing after the ethylene glycol treatment; see the resistance decrease of A-EG-A samples vs. EG-A or A samples of Ink D and E. The resistance of Ink B and C is not greatly increased after 300 h in water.

Biocompatibility studies

The post-processing procedure based on EG and annealing treatment, which results in the lowest degree of leaching and an electrical resistivity well stable over time regardless of whether in a dry or wet state, was used to prepare PEDOT:PSS films on OECT sensor platforms for biocompatibility assays. This procedure involved ethylene glycol which is likely to be retained in the PEDOT:PSS layer and can be released into the culture medium during biocompatibility

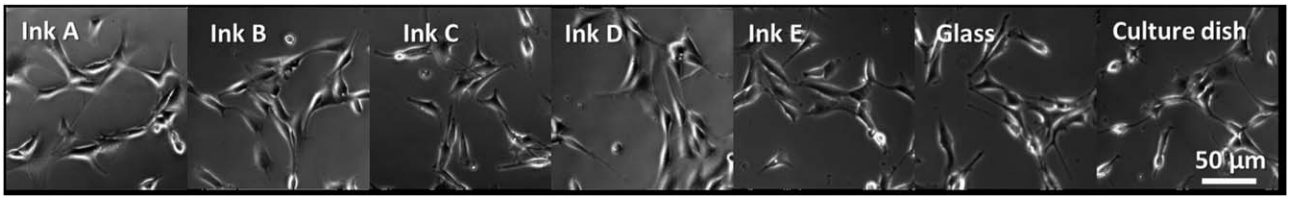

FIGURE 5. Biocompatibility assay of PEDOT:PSS inks: PEDOT formulation spin-coated on OECT substrates was treated with collagen IV. A culture of murine 3T3 fibroblasts was established on them. After two days of culture, coverage with cells and cell morphology was evaluated. Images show typical results out of three biological replicates.

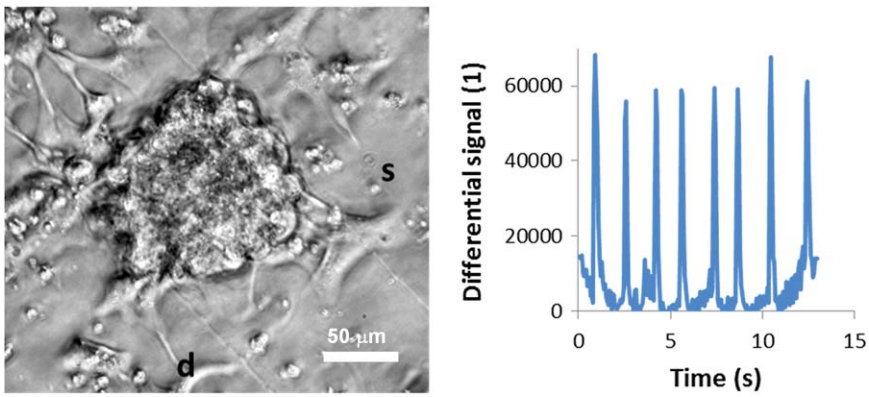

FIGURE 6. The Ink C-based OECT supporting beating murine cardiomyocytes. Left: OECT made using Ink C coated with collagen IV and seeded with embryonic stem cell derived murine cardiomyocytes. s, source electrode; d, drain electrode. Right: Beating of cardiomyocytes quantified by means of video analysis.

assay. This compound, however, did not affect 3T3 fibroblast cell culture in terms of viability and cellular morphology up to a concentration of 1% (V/V) in culture medium (data not shown). Thus, the potential release of small amounts of ethylene glycol out of PEDOT:PSS layer cannot lower the sensor biocompatibility.

In previous sections, we established that a minor proportion of the PEDOT:PSS ink can be released into the aqueous environment. The compounds released could potentially be toxic to cells. Such leaching, however, reaches a plateau within a few minutes (Fig. 2). Hence, it is very likely that the water-soluble components of PEDOT:PSS layers were washed out during the sterilization and subsequent washing steps, indicating that their release into the culture medium during the biocompatibility assay was negligible. Thus, it could be concluded that the most decisive factor affecting the biocompatibility assay was the character of the deposited PEDOT:PSS films.

The biocompatibility was determined using murine 3T3—a cell line commonly applied for such purposes. These cells are anchorage-dependent (i.e., they require a solid support for growth)[22] and are relatively sensitive to exogenous adverse factors. Additionally, they prefer rigid substrates,[23] which matched our purpose as the elastic modulus of most organic semiconductors including PEDOT:PSS is 0.5–20 GPa.[24] Furthermore, they can be scaled up very easily, which makes them a perfect cell line for biocompatibility assays before running experiments with rare and more sensitive cell lines.[16]

Organic semiconductors are generally regarded as biocompatible, as reviewed by recent papers.[25–27] However, in our previous work we have established that a protein coating of PEDOT:PSS is a requisite to achieve high biocompatibility to adherent animal cells. In particular, a coating with collagen IV remarkably increased the ability of PEDOT:PSS to host animal cells.[16] Thus, in order to maximize the biocompatibility of PEDOT:PSS ink-based thin films on OECT sensor platforms, such coating was carried out in this study as well.

All collagen IV-coated PEDOT:PSS inks showed coverage with cells at the level of the culture dish. Furthermore, the morphology of cells grown on collagen IV-coated PEDOT:PSS was very similar to that of cells grown on the culture dish (Fig. 5). This indicates a high level of biocompatibility of collagen IV-coated PEDOT:PSS inks.

The Ink C-based PEDOT:PSS layer showed low electrical resistance and stability over a long time (Figs. 1 and 4) as well as low dissolution in an aqueous environment (Fig. 4). Thus, we have explored its biocompatibility in detail. The culture of 3T3 fibroblasts grown on this material showed a viability $84 \pm 11\%$ (mean value $\pm$ SD, $N = 3$) compared to control cells grown on the culture plastics ($100 \pm 12\%$; mean value $\pm$ SD, $N = 3$) as determined by the ATP assay. Having established the high biocompatibility of Ink C upon a collagen IV coating, it was checked to see if an OECT device based on Ink C can support murine cardiomyocytes. These cells are generally more sensitive to adverse factors than 3T3 fibroblasts. We observed that these cells were able to attach to the active element of the sensor. Moreover, spontaneously beating clusters were formed, indicating good physiological status for the cells (Fig. 6, Supporting Information, video).

To date most electrical sensors to determine cardiomyocyte physiology have been based on inorganic conductive materials[28–30] and references herein. A breakthrough article describing the use of a PEDOT:PSS-based OECT for this purpose has recently been published.[31] However, that sensor was prepared by employing photolithography. The PEDOT:PSS inks that are included in our study are ink-jet ready, which improves the scalability of the production procedure greatly and reduces the potential cost of the sensor and overall analysis. This could have a major impact on biomedical research.

CONCLUSIONS

We can conclude that the thin films prepared from PEDOT:PSS inks without an additive (ethylene glycol in this work) will not provide sufficient conductivity and stability for use in biomedical applications as exemplified by the OECT-based bioelectrical sensor platform tested in this work. The observed high resistance for as-cast films was

successfully reduced by the treatment proposed in this work: additional exposure to ethylene glycol in combination with thermal annealing provided thin films with electrical resistance and its stability in aqueous media comparable to the inks with additives. However, the long-term test of thin films exposed to water revealed that addition of ethylene glycol in the ink rather than subsequent treatment of the films is favorable. While the initial resistances are comparable, long-term exposure to water (12 days) is much less harmful to the films prepared from inks with ethylene glycol included directly in the ink.

Also, the resistance toward water is mostly poor for as-cast films. We observed leaching of the PEDOT:PSS into the water above the films for all of the studied PEDOT:PSS ink formulations. After an initial release, the films are stabilized within a few minutes. The films' stability was greatly improved using treatment with ethylene glycol and annealing. Both procedures can decrease the leaching and improve the films' electrical properties and resistance against water, but the actual influence differs for the ink formulations. Generally, to obtain stable films, mainly annealing is necessary. After 12 days, non-annealed films were completely released from the surface. The best electrical stability was achieved using a combination of thermal annealing followed by ethylene glycol treatment and again thermal annealing.

All PEDOT:PSS inks included in the study were found to be highly biocompatible upon collagen IV coating. Indeed their biocompatibility reached the level of standard cell culture plastics. Such results indicate that any PEDOT:PSS ink formulation which provides films stable in an aqueous environment could reach high biocompatibility upon suitable coating. Thus, the main requisites for selection of a PEDOT:PSS ink for production of films for biointerfaces for biomedical research are good conductivity and aqueous stability of the films. These parameters can be greatly improved by the procedures described here. The minor leaching of PEDOT:PSS films documented in this work cannot in principle affect the cells since the water-soluble compounds are well washed during their preparation for the cell culture. Furthermore, the obtained high level of biocompatibility after collagen IV coating allowed establishment of a culture of stem cell-derived cardiomyocytes in a sensor prototype. A regular spontaneous beating of these cells documented their good physiological status. Such achievements open the door to construction of printed bioelectronic devices for biomedical applications as exemplified here by an OECT-based sensor for determination of the cardiomyocytes' physiology.

REFERENCES

1. Sun K, Zhang S, Li P, Xia Y, Zhang X, Du D, Isikgor FH, Ouyang J. Review on application of PEDOTs and PEDOT:PSS in energy conversion and storage devices. J Mater Sci Mater Electron 2015;26: 4438.
2. Ho S, Liu S, Chen Y, So F. Review of recent progress in multilayer solution-processed organic light-emitting diodes. J Photon Energy 2015;5:057611.
3. Shi H, Liu C, Jiang Q, Xu J. Effective approaches to improve the electrical conductivity of PEDOT:PSS: A review. Adv Electron Mater 2015;1:1500017.
4. Ates M. A review study of (bio)sensor systems based on conducting polymers. Mater Sci Eng C 2013;33:1853–1859.
5. Bernard C, Simon DT, Malliaras G. Bioelectronics for interfacing with the brain. In: Bredas JL, Marder SR, editors. The WSPC Reference on Organic Electronics: Organic Semiconductors, Volume 2: Fundamental Aspects of Materials and Applications. New Jersey: World Scientific; 2016. p 345–368.
6. Tybrandt K, Larsson KC, Kurup S, Simon DT, Kjall P, Isaksson J, Sandberg M, Jager EWH, Richter-Dahlfors A, Berggren M. Translating electronic currents to precise acetylcholine-induced neuronal signaling using an organic electrophoretic delivery device. Adv Mater 2009;21:4442–4446.
7. Pillay V, Tsai T-S, Choonara YE, du Toit LC, Kumar P, Modi G, Naidoo D, Tomar LK, Tyagi C, Ndesendo VMK. A review of integrating electroactive polymers as responsive systems for specialized drug delivery applications. J Biomed Mater Res A 2014;102:2039–2054.
8. Søndergaard RR, Hösel M, Krebs FC. Roll-to-roll fabrication of large area functional organic materials. J Polym Sci Pol Phys 2013;51:16–34.
9. Khan S, Lorenzelli L, Dahiya RS. Technologies for printing sensors and electronics over large flexible substrates: A review. IEEE Sens J 2015;15:3164–3185.
10. Salyk O, Víteček J, Omasta L, Šafaříková E, Stříteský S, Vala M, Weiter M. Organic electrochemical transistors microplate for real-time cell culture monitoring. Appl Sci 2017;7:998.
11. Groth T, Falck P, Miethke R. Cytotoxicity of biomaterials—Basic mechanisms and in vitro test methods: A review. ATLA Altern Lab Anim 1995;23:790–799.
12. Rosenbluth SA, Weddington GR, Gues WL, Autian J. Tissue culture method for screening toxicity of plastic materials to be used in medical practice. J Pharm Sci 1965;54:156–159.
13. Webb K, Hlady V, Tresco PA. Relative importance of surface wettability and charged functional groups on NIH 3T3 fibroblast attachment, spreading, and cytoskeletal organization. J Biomed Mater Res 1998;41:422.
14. Wilson CJ, Clegg RE, Leavesley DI, Pearcy MJ. Mediation of biomaterial–cell interactions by adsorbed proteins: A review. Tissue Eng 2005;11:1–18.
15. Jonas F, Krafft W. European Patent EP 440 957 (Bayer AG), 1990.
16. Šafaříková E, Švihálková Šindlerová L, Stříteský S, Kubala L, Vala M, Weiter M, Víteček J. Towards highly biocompatible organic semiconductors interfaces for bioelectronic devices. Sensor Actuat B Chem 2018 (accepted).
17. Radaszkiewicz K, Sýkorová D, Karas P, Kudová J, Kohút L, Binó L, Večeřa J, Víteček J, Kubala L, Pacherník J. Simple non-invasive analysis of embryonic stem cell-derived cardiomyocytes beating in vitro. Rev Sci Instrum 2016;87:024301–024301.
18. Bernards DA, Malliaras G. Steady-state and transient behavior of organic electrochemical transistors. Adv Funct Mater 2007;17: 3538–3544.
19. Elschner A, Kirchmeyer S, Lovenich W, Merker U, Reuter K. PEDOT: Principles and Applications of an Intrinsically Conductive Polymer. Boca Raton, FL: CRC Press; 2011.
20. Rivnay J, Leleux P, Sessolo M, Khodagholy D, Hervé T, Fiocchi M, Malliaras GG. Organic electrochemical transistors with maximum transconductance at zero gate bias. Adv Mater 2013;25:7010–7014.
21. Ramuz M, Hama A, Huerta M, Rivnay J, Leleux P, Owens RM. Combined optical and electronic sensing of epithelial cells using planar organic transistors. Adv Mater 2014;26:7083–7090.
22. Todaro GJ, Green H. Quantitative studies of the growth of mouse embryo cells in culture and their development into established lines. J Cell Biol 1963;17:299–313.
23. Wong S, Guo WH, Wang YL. Fibroblasts probe substrate rigidity with filopodia extensions before occupying an area. Proc Natl Acad Sci USA 2014;111:17176–17181.
24. Tahk D, Lee HH, Khang D-Y. Elastic moduli of organic electronic materials by the buckling method. Macromolecules 2009;42:7079–7083.
25. Balint R, Cassidy NJ, Cartmell SH. Conductive polymers: Towards a smart biomaterial for tissue engineering. Acta Biomater 2014; 10:2341–2353.
26. Otero TF, Martinez JG, Arias-Pardilla J. Biomimetic electrochemistry from conducting polymers. A review: Artificial muscles, smart

membranes, smart drug delivery and computer/neuron interfaces. Electrochim Acta 2012;84:112–128.
27. Strakosas X, Bongo M, Owens RM. The organic electrochemical transistor for biological applications. J Appl Polym Sci 2015;132:41735.
28. Santoro F, Schnitker J, Panaitov G, Offenhäusser A. On chip guidance and recording of cardiomyocytes with 3D mushroom-shaped electrodes. Nano Lett 2013;13:5379–5384.
29. Braam SR, Tertoolen L, van de Stolpe A, Meyer T, Passier R, Mummery CL. Prediction of drug-induced cardiotoxicity using human embryonic stem cell-derived cardiomyocytes. Stem Cell Res 2010;4:107–116.
30. Doerr L, Thomas U, Guinot DR, Bot CT, Stoelzle-Feix S, Beckler M, George M, Fertig N. New easy-to-use hybrid system for extracellular potential and impedance recordings. J Lab Autom 2015;20:175–188.
31. Yao C, Li Q, Guo J, Yan F, Hsing I. Rigid and flexible organic electrochemical transistor arrays for monitoring action potentials from electrogenic cells. Adv Healthc Mater 2015;4:528–533.