OECT MULTIELECTRODE ARRAY FOR REAL-TIME CELL CULTURE MONITORING

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Abstract

The significant cost of newly developed pharmacological products is due to the toxicity examination performed on animals in pre-clinical trials. Our ultimate goal is to develop a biosensor array to determine acute cardiotoxicity. It capitalizes on the fact that heart muscle cells (cardiomyocytes) show beating *in vitro*. Based on the beating characteristics their pathophysiological status can be deducted. In addition to microscopic imaging, the method of monitoring the action and field potential of synchronously beating cardiomyocyte syncytia on multielectrode arrays (MEA) appears to be a suitable method for the investigation of the condition of cardiac cells. For this purpose, MEA on the basis of organic electrochemical transistors (OECTs) has been developed. The organic polymer PEDOT:PSS was utilized to meet the strict surface biocompatibility demand for a surface in contact with media and living cardiac cells. The MEA consisted of a microplate of a 12x8 chimney - well array with transistors on the bottom for cell cultivation. For the fabrication (a screen printing method) primarily a contact field and an organic polymer was used. The device was tested in a simulation mode for the response of the drain current to the voltage pulses applied to the gate electrode up to 100 μV. The response to such gate voltage renders the device sensitive enough to detect the electrical signal of beating cardiomyocytes. Further development is directed towards higher time resolution by means of miniaturization of the transistors' channel.

Keywords: OECT, screen printing, organic electrochemical transistor, PEDOT:PSS, microplate, multi-electrode array, cell culture

1. INTRODUCTION

The high electrical conductivity of polyacetylene and other organic compounds with conjugated bonds, discovered by Hideki Shirakawa, Alan Heeger, and Alan MacDiarmid led to intense interest in the use of organics in electronics. This discovery was recognized with the Nobel Prize in Chemistry in 2000 [1]. Especially the following branches saw considerable effort put into related research: photovoltaics, photoluminescence, and bioelectronics as a brand-new field of science. In 1989 the first polymer-based light emitting diode (LED) was discovered using PPV as the emissive layer [2]. However, also the printable properties of organic semiconductors have led to the development of so-called printed electronics.

The biocompatibility of organic semiconductors, predominantly polymers, has made them an appealing choice for application in biosensors and bioactuators in biology and medicine as an ideal biointerface [3]. In 1984 White et al. [4] fabricated the first organic electrochemical transistor (OECT) based on polyaniline (PANI).Heywang and Jonas [5] (1992) reported the electrochemical polymerization of a new conducting polymer, poly(3,4-ethylenedioxythiphene) (PEDOT). Yamato et al. [6] (1995) showed the superiority of Poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT :PSS) in terms of stability against pH and applied potential in comparison with PANI. This polymer became widespread in bioelectronics as active transistor channels and other fields as a conductive thin-film semi-transparent wiring.

Aqueous solution of PEDOT:PSS with various additives such as methanol, ethylene glycol, sorbitol serve as solvents for thin film deposition by spin coating and inks for inkjet printing and screen printing. The additives enhance the resulting conductivity by expanding the coil conformation of the macromolecule backbone into a linear conformation and reorganization of the molecule from benzoid form to the quinoid form [7]. The temporary conductivity of PEDOT:PSS reaches 300 S/cm [8].

Bernards and Malliaras (2007) [9] created a model of OECT electronic function. The electrodes are analogues to inorganic FET, i.e. a gate, source and drain, but it works in aqueous solutions of salts, frequently PBS (Phosphate buffered saline) physiological solution for cultivation of biological materials. The charge transport is mediated by holes moving along the PEDOT backbone. The charge is compensated by dangling bonds in some missing hydrogens on SO_3H groups on parallel PSS - see **Figure 1**. Instead of field-caused accumulation or depletion of charge carriers in the channel, in this case the high mobile holes are substituted by low mobile cations and reversely. Thus, the channel conductance is not modulated by the charge concentration but by the mobility, or we might say by the doping - dedoping process.

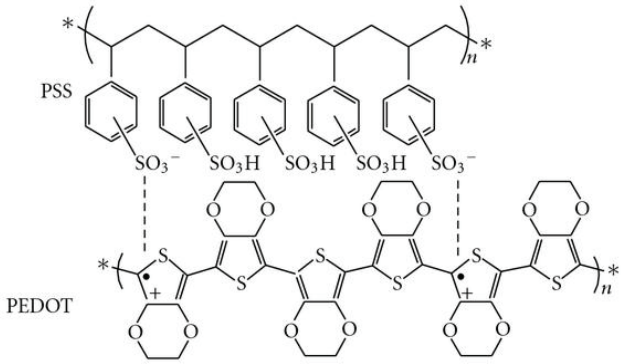

Figure 1 Chemical structure of PEDOT:PSS. The “dot’’ and “plus’’ represent the unpaired electron and positive charge on the PEDOT chain, respectively

Piro et al. (2018) [10] presented in their review a broader insight into the biological application of OECTs. Yao et al. (2014) [11] presented the first study of using OECT arrays for monitoring action potentials from cardiomyocyte-like (i.e. cardiac muscle cell-like) HL-1 cells on both rigid and flexible substrates with improved signal qualities compared to those recorded from grapheme-based electrolyte-gated FETs. Hempel et al. (2016) [12] developed OECT devices in a wafer-scale process and used them as electrophysiological biosensors measuring the electrophysiological activity of the cardiac cell line HL-1. The topography of OECT was further optimized by Rivnay et al. [13] (2015) with respect to enhancing its amplification (transconductance) and frequency range. Proctor et al. (2016) [14] improved the theoretical model of OECTs by introducing a new quantity, namely volumetric capacitance. Tymbrant et al. (2017) [15] completed the theoretical model for OECT and included the ionic double layer at the electrolyte interface and the effects of contact barriers. The biological part of the research is based on research into biocompatibility and cell proliferation [16,17]. The device development is based on our previous experimental work [18].

2. EXPERIMENT

The goal of this study is to investigate the possibility of applying OECTs based on PEDOT:PSS and fabricating a planar printed 12x8 microplate array of transistors. The device (see **Figure 1a**) was constructed on a 96-well polystyrene microplate (chimney-well, no bottom) from Greiner Bio-One GmbH for automated analysis of a multitude of substances. We provided the bottom with the all-printed 12x8 array of Organic Electrochemical Transistors (OECT) based on Poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) polymer (see **Figure 1c**).

In the first stage, the screen-printing method was used. Printed organic electronic technology has brought the possibility of printing biosensor patterns on the biocompatible polymer foils PET (Poly(Ethylene Terephthalate)) or PEN (Poly(Ethylene 2,6-Naphthalate) (Goodfellow Cambridge Ltd), the latter enabling processing at a temperature of up to 170 °C. The bottom substrate was a 0.25 mm thick PEN foil due to better thermal stability during the subsequent annealing processing of the prints. The first printed pattern (with a screen mesh count of 77 threads/cm) of silver paste CB115v2 (©DuPont) Photopolymer and Electronic Materials) creates the contact and conductive field and paths that are 0.18 mm in width (see **Figure 1d**). The foil with the silver pattern was then annealed at 120 °C for fixation.

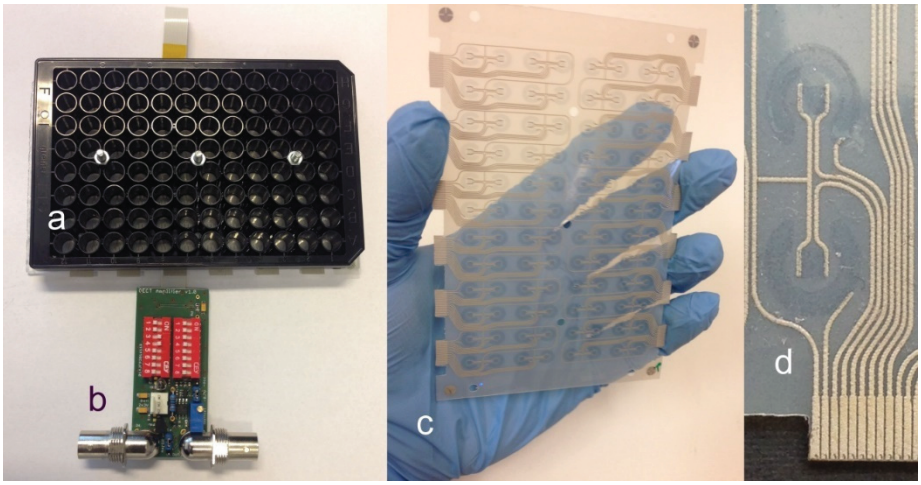

Figure 2 Encapsulated OECT 96 well microplate for electrogenic cell cultivation and investigation (a) with power source-signal amplifier and current/voltage converter (b), foil with functional printed patterns of all OECTs 12x8 array (c) and detail of a couple of OECTs with PEDOT:PSS printed channel and gate electrode (d).

The second pattern of PEDOT:PSS paste Clevios™ S V3 (screen mesh count 140 threads/cm) created a functional OECT gate and channel. The paste was first stirred intensively before printing in order to obtain a lower viscosity and better homogeneity for the resulting film. The silver conductive paste and the polymer patterns were annealed using a hot plate at 150 °C for 15 and 30 minutes respectively in the air to stabilize the film and improve adhesion.

The stirring slightly decreased the final thickness, improved the thickness homogeneity in roughness and waviness measured and the sheet resistance decreased significantly from about 800 Ω/□ to 400 Ω/□. It is considered that intensive stirring acts similarly to alcohol treatment. The system is masked and sealed by the third pattern of Sylgard® 184 (Dow Corning) silicone elastomer (mesh count 120 threads/cm), which insulates the silver conducting paths from the electrolyte environment and also prevents the tested biomaterial from coming into contact with the non-biocompatible parts and layers of the printed device. Other parts and materials were tested for biocompatibility [18]. A coating to increase biocompatibility was done with murine collagen type IV (BD Biosciences, cat. No. 354233) at 10 μg·cm^{-2} according to manufacturer instructions.

Patterning and completing of the microplate followed. The OECT's channel visible in **Figure 2d**, created by a screen-printed rectangular PEDOT:PSS layer on PEN foil, is surrounded by a planar circular gate electrode 6 mm in outer diameter. The rotary symmetry is expected to improve the field distribution. The electrodes are contacted by printed silver conductors 0.2 mm wide. The printed transparent silicone layer covers the surface of the sensing array with the exception of the functional PEDOT:PSS interfacing to the biomaterial and physiological solution. The exposed channel area is of a length of L = 1 mm and a width of W = 1.5 mm, the thickness of the PEDOT:PSS layer was 250 nm on average, and the typical resistance of the channel was

500-700 Ω. The foil was firmly fixed to the polystyrene microplate and bolted down from the bottom by means of a Plexiglas® plate in order to enable easier handling and transparency needed for microscope observations. The second pattern of PEDOT:PSS paste Clevios™ S V3 (screen mesh count 140 threads/cm) created a functional OECT gate and channel. The paste was first stirred.

In the second state the printed circuit technology with a PEN foil of 0.25 mm coated with copper (AKAFLEX® from KREMPEL GmbH) for the substrate was used due to its better conductance and resolution of the lithographically patterned board in comparison with a printed silver paste pattern (see **Figure 3b**). The biocompatibility was provided by the gilding of the conductor paths, and silicon masking was substituted by the SU-8 photoresist patterning with enhanced resolution. The single transistor is depicted in **Figure 3a**. The entire device is assembled as in the previous case. Eight transistors are connected via one Molex connector and sixteen switches to the current/voltage converter - amplifier (see **Figure 2b**), a diagram of which can be found in **Figure 3c**, for further digital processing in a scope or another recording facility.

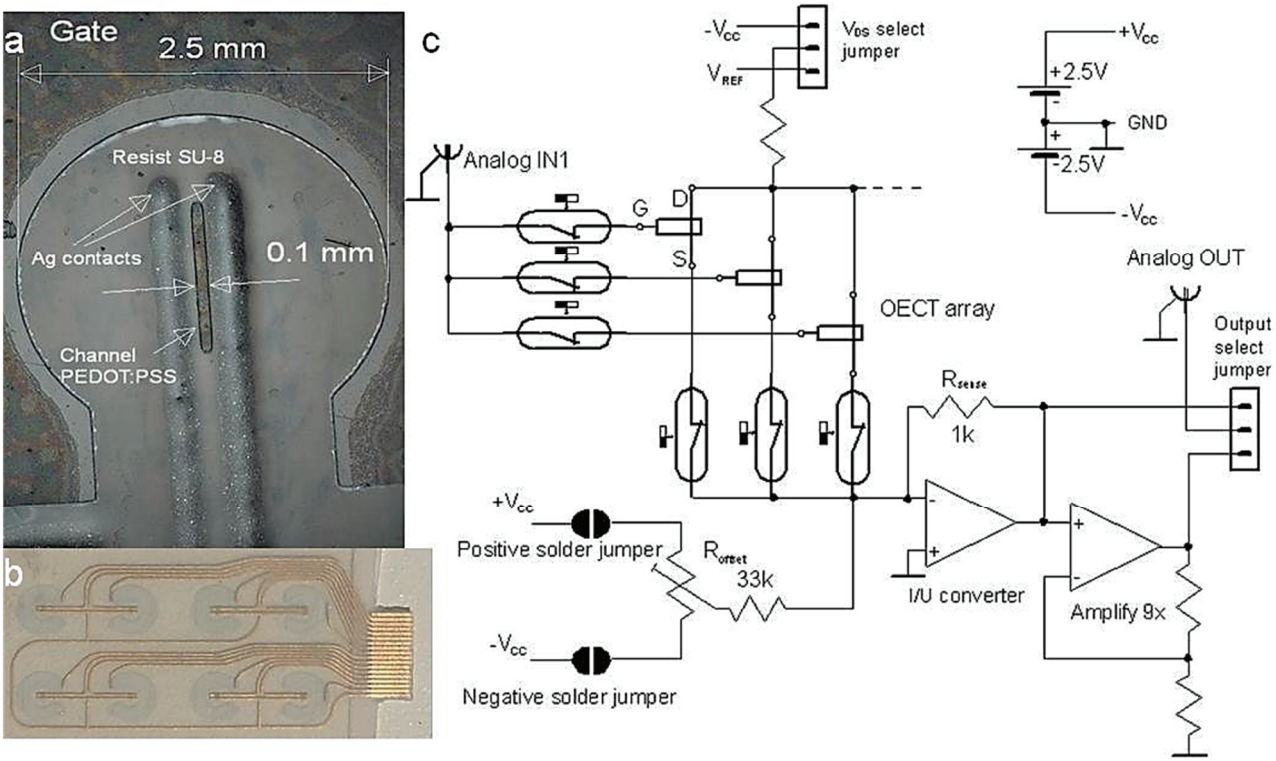

Figure 3 The amplifier - current/voltage converter for testing of the OECTs

3. RESULTS AND DISCUSSION

The device was tested for the OECT output (**Figure 4a**) and transfer (**Figure 4b**) characteristics, where the optimum setpoint with respect to high transconductance and safety against redox reactions was found. Also the frequency/time constant limits were tested by modulating the gate voltage by rectangular pulses 1mV$_{pp}$ and the frequency 1Hz and the responding gate induced drain current I_{Dpp} = 2.5 μA, which results in the transconductance g = 2.5 mS (**Figure 5**). The gate pulses of 250 μV were also reliably detected using FFT analysis. The time constant τ = 0.15 s is given by the serial combination of electrolyte resistance and volumetric capacity of the PEDOT:PSS channel. Other capacities and resistances (gate, electrical double layer - EDL) are neglected.

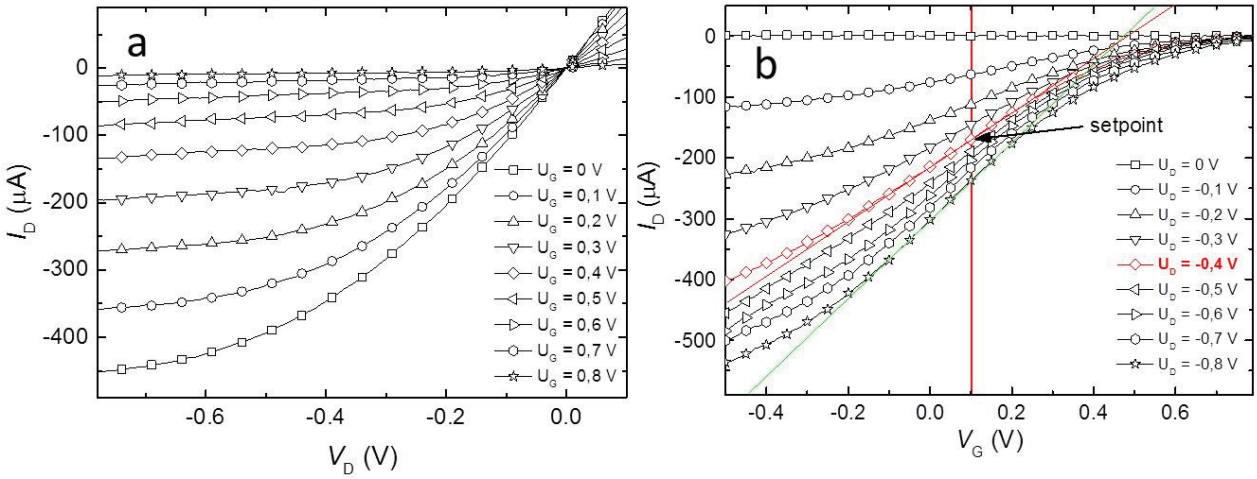

Figure 4 Output and transfer characteristics with the illustration of setpoint and the slope dependent on the drain potential

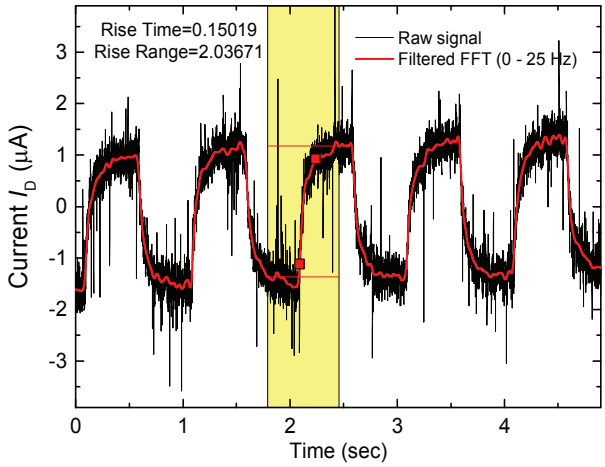

Figure 5 Response of OECT to 1 mV_{pp} gate rectangular signal at setting point V_{DS} = -0.7 V V_{GS} = 0.2 V. The derived transconductance gives g = 2.5 mS and time constant τ = 0.15 s.

4. CONCLUSIONS

The improved screen printing process of photoresist SU-8 masking was optimized and successfully implemented into previously adapted OECT sensors based on PEDOT:PSS and silicon masking [19]. The photolithographic process enabled shortening the channel length down to 100 μm and further and the OECT geometry to adapt on zero gate bias setpoint for maximum signal amplification. Simulating the pulsation of cardiomyocyte cells, the transconductance g = 2.5 mS was achieved with the S/N ratio about 10. The upper-frequency limit 7 Hz was concluded from the OECT gate circuit time constant of 0.15 s. Additional improvement of the device speed and amplification could be obtained by reducing the OECT channel dimensions, mainly by the reducing the PEDOT:PSS volume capacitance by decreasing the channel area and its thickness.

ACKNOWLEDGEMENTS

This work was supported by Czech Science Foundation via project No. 17-24707S, research infrastructure was supported by projects MŠMT No. LO1211 and J. Ehlich was supported by the Faculty of Chemistry at BUT under project No. FCH-S-17-4685.

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