Materials Science & Engineering C 81 (2017) 84–89

Contents lists available at ScienceDirect

Materials Science & Engineering C

journal homepage: www.elsevier.com/locate/msec

Short communication

# Simultaneous monitoring of single cell and of micro-organ activity by PEDOT:PSS covered multi-electrode arrays

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ARTICLE INFO

*Keywords:*
Bioelectronics
Biosensor
PEDOT:PSS
Diabetes
Islets
Pancreatic beta-cells

ABSTRACT

Continuous and long-term monitoring of cellular and micro-organ activity is required for new insights into physiology and novel technologies such as Organs-on-Chip. Moreover, recent advances in stem cell technology and especially in the field of diabetes call for non-invasive approaches in quality testing of the large quantities of surrogate pancreatic islets to be generated. Electrical activity of such a micro-organ results in single cell action potentials (APs) of high frequency and in low frequency changes in local field potentials (slow potentials or SPs), reflecting coupled cell activity and overall organ physiology. Each of them is indicative of different physiological stages in islet activation. Action potentials in islets are of small amplitude and very difficult to detect. The use of PEDOT:PSS to coat metal electrodes is expected to reduce noise and results in a frequency-dependent change in impedance, as shown here. Whereas detection of high-frequency APs improves, low frequency SPs are less well detected which is, however, an acceptable trade off in view of the strong amplitude of SPs. Using a dedicated software, recorded APs and SPs can be automatically diagnosed and analyzed. Concomitant capture of the two signals will considerably increase the diagnostic power of monitoring islets and islet surrogates in fundamental research as well as drug screening or the use of islets as biosensors.

## 1. Introduction

Continuous and long-term monitoring of cellular and micro-organ activity is required for new insights into physiology, constitutes a mainstay of novel technologies such as Organs-on-Chip and will also open new therapeutic possibilities in biomedicine [1]. Moreover, with the recent advances in stem cell technology and ensuing de-novo creation of organs, non-invasive approaches are required to improve current protocols. In the same vein, simultaneous monitoring of individual cells as well as organ activity is necessary for quality testing of the large amount of surrogate organs to be generated before their use for therapeutic purposes. These issues have also become important in the diabetes field, especially for type 1 diabetes which is characterized by a loss of islet β-cells [2].

Ideally, continuous monitoring should be non-invasive and non-destructive, and not require changes in the genetic repertoire or addition of chemical probes. Recording electrical membrane potentials from excitable cells, which include not only neurons and muscle but also endocrine cells, should offer a convenient approach, especially via non-invasive extracellular electrodes. Such a non-invasive approach also offers the possibility to perform long-term experiments more in line with physiology. Indeed, physiological activation of islets occurs during an approximately 2 h long digestion period with a specific and defined pattern [3]. The use of electronics avoids heat-generation or bleaching that may damage biological samples, as encountered in optical means. Electric potentials are created by changes in ion fluxes. Their information content is high as changes in membrane potentials are often the first integrative signal of physiological regulations [4]. Excitable organs or cell clusters generate two types of electrical signals: short-lived high frequency action potentials (AP) that reflect single cell depolarization and longer lived changes in field potentials, the low frequency slow potentials (SP). The latter reflects the integrative electrical

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http://dx.doi.org/10.1016/j.msec.2017.07.028
Received 9 May 2017; Received in revised form 20 June 2017; Accepted 18 July 2017
Available online 19 July 2017

activity at the organ or micro-organ level. Ideally, both should be recorded to obtain information about the activity of single cells, which may be of different type and behavior, and of the whole (micro) organ that provides information on a higher level of physiological integration. Clearly simultaneous capture and analysis of both events avoids complex and potentially biased mathematical post-hoc modeling to deduce physiological intra-organ cell coupling and thus presents an important asset for fundamental approaches as well as the use of micro-organs as biosensor.

APs recorded by extracellular electrodes are of considerable amplitude in neurons or cardiomyocytes. The situation is different in other excitable cells such as in the islets of the endocrine pancreas. In this micro-organ α- and β-cells produce action potentials, but only β-cells are electrically coupled and generate SPs [5]. Whereas SPs are robust, APs are often small and very difficult to detect in endocrine cells when using electrodes in standard materials such as Titanium Nitride (TiN) and Platinium [5–7]. Some elegant approaches have been published involving a sophisticated geometry for extracellular electrodes with electrodes in form of micro- or submicrometric mushrooms that are engulfed by the plasma membrane. This provides some improvements but do not enhance detection of APs [8]. Detection can be improved by filtering, but this is ultimately not satisfactory [9]. We therefore searched for means to improve detection of islet cells' APs at the electrode level while preserving SP detection. One possibility would be to increase electrode size, but this reduces spatial discrimination and endocrine micro-organs often have a diameter of 50 μm, severely limiting this factor. Conducting polymers such as poly(3,4-ethylenedioxythiophene), complexed with polystyrene sulfonate (PEDOT:PSS) are known to decrease electrode impedance and achieve higher signal-to-noise ratios [10]. Clearly, this may favorably influence AP detection.

## 2. Experimental

### 2.1. Device fabrication

Devices were fabricated as previously reported [11]. A 26 mm × 76 mm glass slide was used as a substrate after thorough cleansing in a 1:1 (vol/vol) acetone/isopropanol solvent mixture. The gold electrodes were patterned on top of the glass slide in a standard photolithographic technique with the use of S1813 photoresist, subsequent UV light exposure and development in MF-26 developer. The resulting gold electrodes were 100 nm thick while a 10 nm thick Cr layer between them and the substrate was chosen to act as an adhesive promoter. Both metals were deposited via standard metal evaporation. Thereafter, two layers of Parylene C (with the second serving as a sacrificial one in a later stage) were deposited for the device encapsulation with an antiadhesive soap layer in between consisting of Micro-90 soap (1% v/v solution in bidistilled water; Sigma Aldrich, Z281506). A second photolithography step was used to pattern the electrode active area with the use of AZ 9260 photoresist and development in AZ developer. Reactive Ion Etching with $O_2$ plasma created openings in Parylene C before the PEDOT: PSS suspension was spun on the device. A final peel-off step defined the conducting material-covered electrode area by the removal of the second sacrificial Parylene C layer. The devices were hard baked for 60 min at 140 ° C and immersed in bidistilled water overnight for the removal of any low molecular weight compounds of the PEDOT:PSS dispersion. The PEDOT: PSS formulation used is as follows: 38 mL of PEDOT:PSS aqueous dispersion (Clevios PH − 1000) were mixed with 2 mL of ethylene glycol (for conductivity enhancement), 50 μL of 4-dodecylbenzenesulfonic acid (DBSA) that helps the film formation and 0.4 mL of 3-methacryloxypropyltrimethoxysilane (GOPS) which is a surface adhesion promoter and a polymer cross-linking agent that enhances film stability in aqueous environments. Commercial devices were obtained from Multichannel Systems (Tübingen, Germany).

### 2.2. Islet isolation and cell culture

Islet cells were obtained from mice as published [9,12]. Before cell culturing the devices were plasma treated for 2 min to render them hydrophilic (the plasma treatment was 9.82 W/L–27.5 W in a 2.8 L chamber) as described [7] and islets seeded as described [5]. Human islets were isolated from non-diabetic donors at the Cell Isolation and Transplantation Center (Geneva University Hospital) and handled as described [5].

### 2.3. Device characterization and electrophysiological recordings

Devices were characterized with a potentiostat (Autolab PGSSTAT128N) in a three electrode configuration in 0.1 M NaCl solution. The PEDOT:PSS covered electrodes served as the working electrode while a Pt and Ag/AgCl electrode were used as counter and reference electrode, respectively. Measurements were performed with an INTAN RHD2132 32-channels amplifier chip with unipolar inputs (Intan Technologies) at a sampling rate of 10 kHz.

### 2.4. Data processing and spectral analysis

Processing was performed offline using a custom Matlab program (Mathworks, Cambridge, UK). It covers AP and SP detection, frequency and amplitude measurements and generates processed data and image files for visual control. Regarding AP detection, a band-pass Butterworth filter was used to isolate AP waveforms. Traditionally, wavelet filters are used for this purpose, since they increase the signal-to-noise ratio of the AP signal, but they also tend to modify the APs' amplitudes, which would have altered our measurements. APs were detected when the band-pass filtered signal exceeded a threshold set at 2.9 times the standard deviation of the high-pass part of the signal for each electrode. Digital blanking was used to avoid multiple detections of the same AP: subsequent detections were ignored for 75 ms. Each AP's peak-to-peak amplitude was individually measured from the filtered signal, in a 50 ms window, starting 10 ms before the detection. In parallel, SPs were isolated using a third-order band-pass 0.1-1 Hz Butterworth filter. SPs were detected by finding their individual maxima and minima, discriminated using an amplitude tolerance of 8 μV that avoided the detection of small ripples in the filtered signal. The extrema values were stored and used to measure the peak-to-peak amplitude of each SP. Frequency measurements on both APs and SPs were performed using second order IIR filters with a time constant of 25.6 s. The total number of events detected here were for APs 12,563 (TiN, 61 electrodes) and 4364 (PEDOT:PSS, 14 electrodes); for SPs 5286 (TiN, 59 electrodes) and 891 (PEDOT:PSS, 14 electrodes). Frequency repartition of noise was estimated by applying Fast Fourier Transforms on 600 s recordings of uncovered electrodes for both PEDOT:PSS and TiN technologies. This yielded the power spectral density of the noise in dBμV/Hz, which indicates how noise is distributed frequency-wise and consequently which portions of the biological signal are more impacted by noise.

## 3. Results and discussion

A corresponding device was assembled (Fig. 1a for scheme) and its electric characteristics determined in relation to commercial titanium nitride (TiN) electrodes or gold electrodes. As expected, PEDOT:PSS coverage considerably improved the performance of gold electrodes in terms of impedance and the related capacitance (Fig. 1b and c) which is reflected by the known improvement in signal recording. The comparison of TiN- and gold/PEDOT:PSS electrodes revealed a less pronounced difference and was dependent on frequency. Whereas both types of electrodes behaved similarly at frequencies between 1 and 300 Hz, differences were observed below and above this range for impedance and even more marked for capacitance. The expected range for