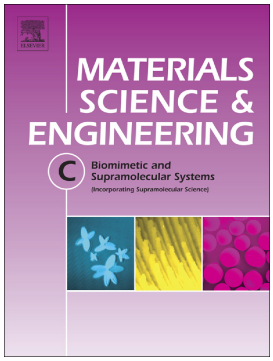


Simultaneous monitoring of single cell and of micro-organ activity by PEDOT:PSS covered multi-electrode arrays

Dimitiros A. Koutsouras, Romain Perrier, Ariana Villaroel-Marquez, Antoine Pirog, Eileen Pedraza, Eric Cloutet, Sylvie Renaud, Matthieu Raoux, George G. Malliaras, Jochen Lang

PII: S0928-4931(17)31785-X
DOI: doi: 10.1016/j.msec.2017.07.028
Reference: MSC 8177

To appear in: *Materials Science & Engineering C*

Received date: 9 May 2017
Revised date: 20 June 2017
Accepted date: 18 July 2017

Please cite this article as: Dimitiros A. Koutsouras, Romain Perrier, Ariana Villaroel-Marquez, Antoine Pirog, Eileen Pedraza, Eric Cloutet, Sylvie Renaud, Matthieu Raoux, George G. Malliaras, Jochen Lang , Simultaneous monitoring of single cell and of micro-organ activity by PEDOT:PSS covered multi-electrode arrays, *Materials Science & Engineering C* (2017), doi: 10.1016/j.msec.2017.07.028

Title

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

*Author(s), and Corresponding Author(s)**

Dimitiros A. Koutsouras [1], Romain Perrier [2], Ariana Villaroel-Marquez [2,3], Antoine Pirog [4], Eileen Pedraza [2], Eric Cloutet [3], Sylvie Renaud [4], Matthieu Raoux [2], George G. Malliaras [1], Jochen Lang [2*]*

D.A. Koutsouras and G.G. Malliaras,
Department of Bioelectronics, Ecole Nationale Supérieure des Mines, CMP-EMSE, 13541 Gardanne, France
malliaras@emse.fr

R. Perrier, A. Villaroel Marquez, E. Pedraza, M. Raoux and J. Lang
Chimie et Biology des Membranes et Nano-Objets (CBMN), UMR CNRS 5248, Université de Bordeaux, 18 Av Geoffroy St Hilaire, 33600 Pessac, France
jochen.lang@u-bordeaux.fr

A. Villaroel-Marquez and E. Cloutet
Laboratoire de Chimie des Polymères Organiques (LCPO), UMR CNRS 5629, Université de Bordeaux, 33600 Pessac, France

A. Pirog and S. Renaud
Laboratoire d'Intégration de Materiaux aux Systèmes (IMS), UMS CNRS 5218, Université de Bordeaux and Institut Polytechnique de Bordeaux, 33400 Talence, France

DAK et RP contributed equally
ORCID Jochen Lang: orcid.org/0000-0001-6847-5641

Keywords: Bioelectronics; biosensor; PEDOT:PSS; diabetes; islets

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 for 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 two hours long digestion period with a specific and defined pattern [3]. The use of electronics avoid 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 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 modelling 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 from of micro- or submicrometric mushrooms that are engulfed by the plasma membrane. This provides some improvement 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 x 76 mm glass slide was used as a substrate after thorough cleansing in a 1:1 (vol/vol) acetone/isopropanol solvent. 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 minutes 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 enhance 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 minutes 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 10kS/s.

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 50ms window, starting 10ms before the detection. In parallel, SPs were isolated using a third-order band-pass 0.1-1Hz 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 12563 (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 (**Figure 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 (**Figure 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 SPs (0.1-1 Hz) and APs (>300 Hz) are shaded in grey in Figure 1 b and c. Thus the behavior of the two types of electrodes predicts an improved signal/noise ratio for PEDOT:PSS covered gold electrodes in the high frequency range, typical for APs, and a less favorable outcome at low frequencies such as those found for SPs.

As SPs are rather robust and of considerable amplitude, we expected the trade-off in the low frequency range to be acceptable in view of an improved recognition of APs. We therefore recorded pancreatic β-cell activity from rodent or human islets in-vitro (**Figure 2**). As shown in **Figure 2a**, these extracellular electrodes record the sum of changes in ion fluxes, mainly Na^+, K^+ and Ca^{2+}, brought upon by the electrical activity of the cells subsequent to changes in ion channel activities. Their activity is regulated in islet β-cells by the ambient glucose concentration and changes in circulating hormones from the gut or stress mediators such as adrenalin. Metabolism of glucose, lipids or certain amino acids leads to the intracellular increase in metabolic coupling factors, such as ATP, that leads to the closure of ATP-sensitive potassium channels and subsequent membrane depolarization with ensuing opening of voltage-dependent calcium channels [13]. Electrical activity of islets is biphasic and proceeds first mainly through single cell activity with action potentials and subsequently results in a second phase, vital for physiological glucose homeostasis, which is characterized by pronounced coupling between cells [14]. Stress or gut hormones further regulate ion channel activity via classical cAMP or PLC pathways [15]. Changes in ion fluxes therefore constitute the first integrative read-out combining nutrient levels and the status of the entire organism, and finally lead to the secretion of the hormone insulin to adapt glucose homeostasis to the physiological needs.

Figure 2b depicts islet cell clusters seeded on PEDOT:PSS covered gold electrodes and exposed to different stimuli. Representative recordings of an electrode covered by islets (electrode 1) are given in **Figure 2c**. Changing glucose levels from a low normoglycemic concentration of 3 mM to a hyperglycemic level of 15 mM induces a strong electrical

response. Addition of the stress hormone adrenalin to 15 mM glucose reduces transiently the islet activity as expected. An even stronger level of activity can be elicited by the subsequent addition of the drug glibenclamide, used in the treatment of type 2 diabetes and known to directly close K_{ATP} channels. The addition of nifedipine, a blocker of voltage dependent calcium channels, strongly inhibits the electrical activity evoked by 15 mM glucose indicating that calcium ion fluxes contribute considerably to the changes in field potentials. These observations are in line with the activities described previously by us using TiN or platinium electrodes [5, 7]. To further illustrate the nature of the electrical signals recorded, magnifications of the recording are given in the lower panel of Figure 2c. They demonstrate the presence of low frequency waves, the SPs, and small superimposed activities of high frequencies, the APs. To better demonstrate the presence of the two types of events, we have done some filtering on the traces in presence of glucose and glibenclamide. Whereas a 2 Hz low-pass filter sorts mainly the SPs (red), a 2-700 Hz band pass filter cuts out the SPs and demonstrates the presence of APs (blue). Figure 2d shows the activity recorded from another electrode (electrode 2) that was not covered by islets and consequently no specific signal was recorded.

Although mouse islets provide a useful model, it has become clear recently that human islets show a number of differences also in terms of electrophysiology [3]. We have therefore also tested human islets, obtained post-mortem from donors, and some results are shown in **Figure 2e**. Recordings shown were obtained from two different donors. Islets of donor 1 exhibit prominent SPs with some APs and activity can be blocked by the use of adrenalin. Islet activity of donor 2 was characterized by the presence of very marked APs that were fully sensitive to nifedipine. Also both responded to glucose and inhibitors, this suggests a marked heterogeneity between donors. Indeed considerable variances have been described for a number of biological responses of human islets and reflect probably their diverse genetic background and history as compared to inbred rodent strains [16].

Collectively these experiments suggest that APs may be easier to capture using PEDOT:PSS covered electrodes. We therefore directly and quantitatively compared those electrodes with classical TiN electrodes. To avoid bias and provide a rapid mean we devised an offline Matlab routine as current commercial programs are rather time consuming and not able to reliably distinguish between SPs and APs of small amplitude. We have previously described approaches that determine frequencies of APs or SPs, but not amplitudes. As depicted in **Figure 3a**, input data are filtered, detection thresholds are set and the read-out provides frequency and amplitude for both types of events. As demonstrated in **Figure 3b**, this allows rapid detection of SPs and APs from traces, such as here during stimulation by glucose. Statistical evaluation of TiN vs Au/PEDOT:PSS recordings revealed interesting differences (**Figure 3c**). Au/PEDOT:PSS clearly captured a higher number of APs but lost some SPs. Interestingly, amplitudes of APs were comparable between TiN and Au/PEDOT:PSS indicating the increase in captured events does not reflect an artefact and

are most likely due to lower noise levels. In contrast, amplitudes of SPs were largely reduced.

The initial characterization of impedance and capacitance (Figure 1) was done under ideal conditions, only with the potentiostat, as opposed to real working conditions, i.e. with a complete recording set-up (acquisition device, heating systems, amplifiers, computers, power supplies, and culture solutions). To evaluate whether this seemingly frequency dependent behavior of the electrodes can be found also under real working conditions, we determined the power spectrum of the two types of electrodes using recordings from electrodes that had not been covered by islets. As can be seen in Figure 3d, noise density is higher in the SP region (0.1-1 Hz) in PEDOT:PSS electrodes, meaning that SPs will have a lower signal-to-noise ratio, impeding their detection. On the opposite, noise density in the AP region (400-5000 Hz) is lower in PEDOT:PSS electrodes, improving AP signal-to-noise ratio and detection. We have again similar frequency-dependent characteristics indicating that indeed noise is more prominent at low frequencies in PEDOT:PSS electrodes but more favorable at high frequencies. Given the higher robustness of SPs in comparison to APs, signal-to-noise ratio loss in low-frequencies is acceptable. While AP detection reliability typically relies on signal processing [9], PEDOT:PSS covered electrodes constitute an upstream material improvement.

4. Conclusion

In conclusion, the frequency-dependent behavior of PEDOT:PSS permits simultaneous recording of single cell and micro-organ activity in the rodent and human endocrine system in a non-invasive system using native biological preparations. As shown, the two types of signals can be reliably distinguished by a novel software script under Matlab, and amplitudes as well as frequencies can be determined automatically. The concomitant capture of the two signals and their analysis with dedicated software considerably increases the diagnostic power of such an approach and avoids complex as well as eventually biased post-hoc in-silico analysis. This quality combined with the non-invasive nature of our approach and the absence of chemical or genetic probes makes it an ideal tool to monitor endocrine systems in the general pursuit to construct an entire animal or human organism on a chip [17, 18] for pharmacological screening or disease modeling. Moreover, such a detection module may provide very useful for the quality control of stem-cell derived islet surrogates and increase the accuracy of bio-inspired sensors for the demand of insulin [19].

Acknowledgements

This work was supported by grants from LABEX Amadeus, SATT Aquitaine and Curie ITN OLIMPIA. The authors D.A. Koutsouras and R. Perrier contributed equally.

References

[1] Luni, C., E. Serena, and N. Elvassore (2014) Human-on-chip for therapy development and fundamental science. Curr Opin Biotechnol 25: p. 45-50. doi: 10.1016/j.copbio.2013.08.015

[2] Melton, D.A. (2016) Applied Developmental Biology: Making Human Pancreatic Beta Cells for Diabetics. Curr Top Dev Biol 117: p. 65-73. doi: 10.1016/bs.ctdb.2015.11.013

[3] Rorsman, P. and M. Braun (2013) Regulation of insulin secretion in human pancreatic islets. Annu Rev Physiol 75: p. 155-179. doi: 10.1146/annurev-physiol-030212-183754

[4] MacDonald, P.E. (2011) Signal integration at the level of ion channel and exocytotic function in pancreatic beta-cells. Am J Physiol Endocrinol Metab 301: p. E1065-1069. doi: 10.1152/ajpendo.00426.2011

[5] Lebreton, F., A. Pirog, I. Belouah, D. Bosco, T. Berney, P. Meda, Y. Bornat, B. Catargi, S. Renaud, M. Raoux, and J. Lang (2015) Slow potentials encode intercellular coupling and insulin demand in pancreatic beta cells. Diabetologia 58: p. 1291-1299. doi: 10.1007/s00125-015-3558-z

[6] Nguyen, Q.V., A. Caro, M. Raoux, A. Quotb, J.B. Floderer, Y. Bornat, S. Renaud, and J. Lang (2013) A novel bioelectronic glucose sensor to process distinct electrical activities of pancreatic beta-cells. Conf Proc IEEE Eng Med Biol Soc 2013: p. 172-175. doi: 10.1109/EMBC.2013.6609465

[7] Pedraza, E., A. Karajic, M. Raoux, R. Perrier, A. Pirog, F. Lebreton, S. Arbault, J. Gaitan, S. Renaud, A. Kuhn, and J. Lang (2015) Guiding pancreatic beta cells to target electrodes in a whole-cell biosensor for diabetes. Lab Chip 15: p. 3880-3890. doi: 10.1039/c5lc00616c

[8] Rabieh, N., S.M. Ojovan, N. Shmoel, H. Erez, E. Maydan, and M.E. Spira (2016) On-chip, multisite extracellular and intracellular recordings from primary cultured skeletal myotubes. Sci Rep 6: p. 36498. doi: 10.1038/srep36498

[9] Raoux, M., Y. Bornat, A. Quotb, B. Catargi, S. Renaud, and J. Lang (2012) Non-invasive long-term and real-time analysis of endocrine cells on micro-electrode arrays. J Physiol 590: p. 1085-1091. doi: 10.1113/jphysiol.2011.220038

[10] Cellot, G., P. Lagonegro, G. Tarabella, D. Scaini, F. Fabbri, S. Iannotta, M. Prato, G. Salviati, and L. Ballerini (2015) PEDOT:PSS Interfaces Support the Development of Neuronal Synaptic Networks with Reduced Neuroglia Response In vitro. Front Neurosci 9: p. 521. doi: 10.3389/fnins.2015.00521

[11] Sessolo, M., D. Khodagholy, J. Rivnay, F. Maddalena, M. Gleyzes, E. Steidl, B. Buisson, and G.G. Malliaras (2013) Easy-to-Fabricate Conducting Polymer Microelectrode Arrays. Advanced Materials 25: p. 2135-2139. doi: 10.1002/adma.201204322

[12] Raoux, M., P. Vacher, J. Papin, A. Picard, E. Kostrzewa, A. Devin, J. Gaitan, I. Limon, M.J. Kas, C. Magnan, and J. Lang (2015) Multilevel control of glucose homeostasis by adenylyl cyclase 8. Diabetologia 58: p. 749-757. doi: 10.1007/s00125-014-3445-z

[13] MacDonald, P.E., J.W. Joseph, and P. Rorsman (2005) Glucose-sensing mechanisms in pancreatic beta-cells. Philos Trans R Soc Lond B Biol Sci 360: p. 2211-2225. doi: 10.1098/rstb.2005.1762

[14] Johnston, N.R., R.K. Mitchell, E. Haythorne, M.P. Pessoa, F. Semplici, J. Ferrer, L. Piemonti, P. Marchetti, M. Bugliani, D. Bosco, E. Berishvili, P. Duncanson, M. Watkinson, J. Broichhagen, D. Trauner, G.A. Rutter, and D.J. Hodson (2016) Beta Cell Hubs Dictate Pancreatic Islet Responses to Glucose. Cell Metab 24: p. 389-401. doi: 10.1016/j.cmet.2016.06.020

[15] Campbell, J.E. and D.J. Drucker (2013) Pharmacology, physiology, and mechanisms of incretin hormone action. Cell Metab 17: p. 819-837. doi: 10.1016/j.cmet.2013.04.008

[16] Blodgett, D.M., S.D. Redick, and D.M. Harlan (2016) Surprising Heterogeneity of Pancreatic Islet Cell Subsets. Cell Syst 3: p. 330-332. doi: 10.1016/j.cels.2016.10.009

[17] Zhang, Y.S., J. Aleman, S.R. Shin, T. Kilic, D. Kim, S.A. Mousavi Shaegh, S. Massa, R. Riahi, S. Chae, N. Hu, H. Avci, W. Zhang, A. Silvestri, A. Sanati Nezhad, A. Manbohi, F. De Ferrari, A. Polini, G. Calzone, N. Shaikh, P. Alerasool, E. Budina, J. Kang, N. Bhise, J. Ribas, A. Pourmand, A. Skardal, T. Shupe, C.E. Bishop, M.R. Dokmeci, A. Atala, and A. Khademhosseini (2017) Multisensor-integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors. Proceedings of the National Academy of Sciences 114: p. E2293-E2302. doi: 10.1073/pnas.1612906114

[18] Zhang, B. and M. Radisic (2017) Organ-on-a-chip devices advance to market. Lab Chip doi: 10.1039/c6lc01554a

[19] Renaud, S., B. Catargi, and J. Lang (2014) Biosensors in diabetes: how to get the most out of evolution and transpose it into a signal. IEEE Pulse 5: p. 30-34. doi: 10.1109/MPUL.2014.2309577

FIGURES AND LEGENDS

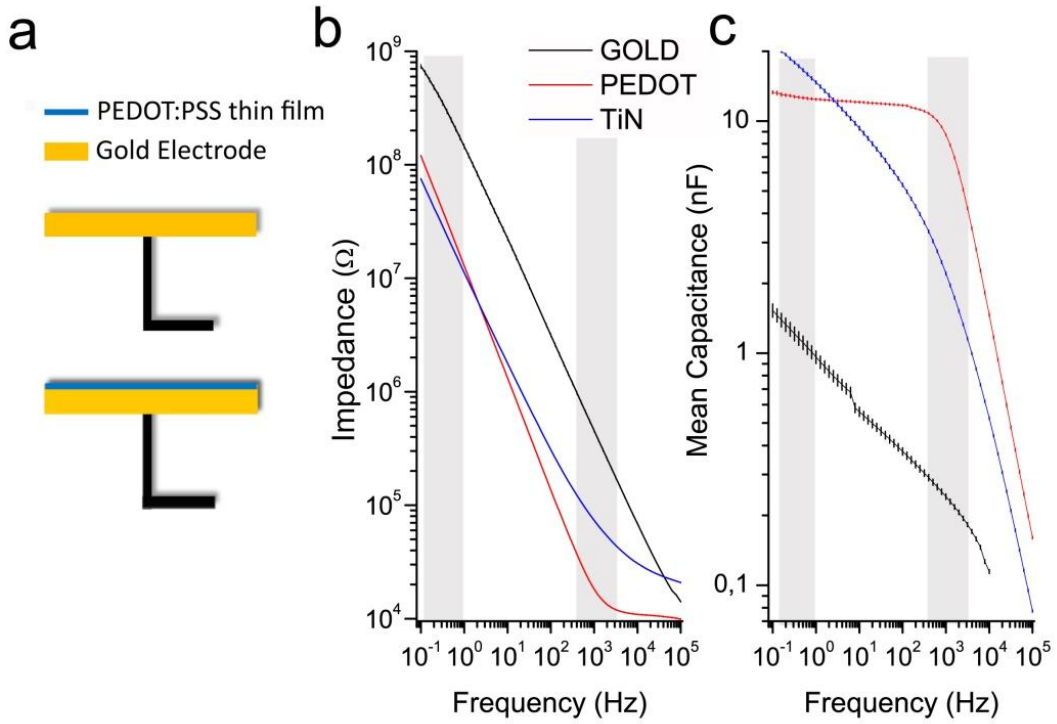


Fig. 1 Electrode impedance and capacitance characteristics

a Scheme of bare or PEDOT:PSS covered electrodes. **b** Absolute impedance as a function of frequency for gold electrodes alone (GOLD) or covered with PEDOT:PSS (PEDOT) or conventional titanium nitride electrodes (TiN). **c** Absolute capacitance as a function of frequency for same electrodes as in b). Given are means and SEM from duplicate measurements of 30 gold, 57 TiN or 9 PEDOT-Au electrodes. The frequency range relevant for slow potentials and action potentials are indicated by a grey background.

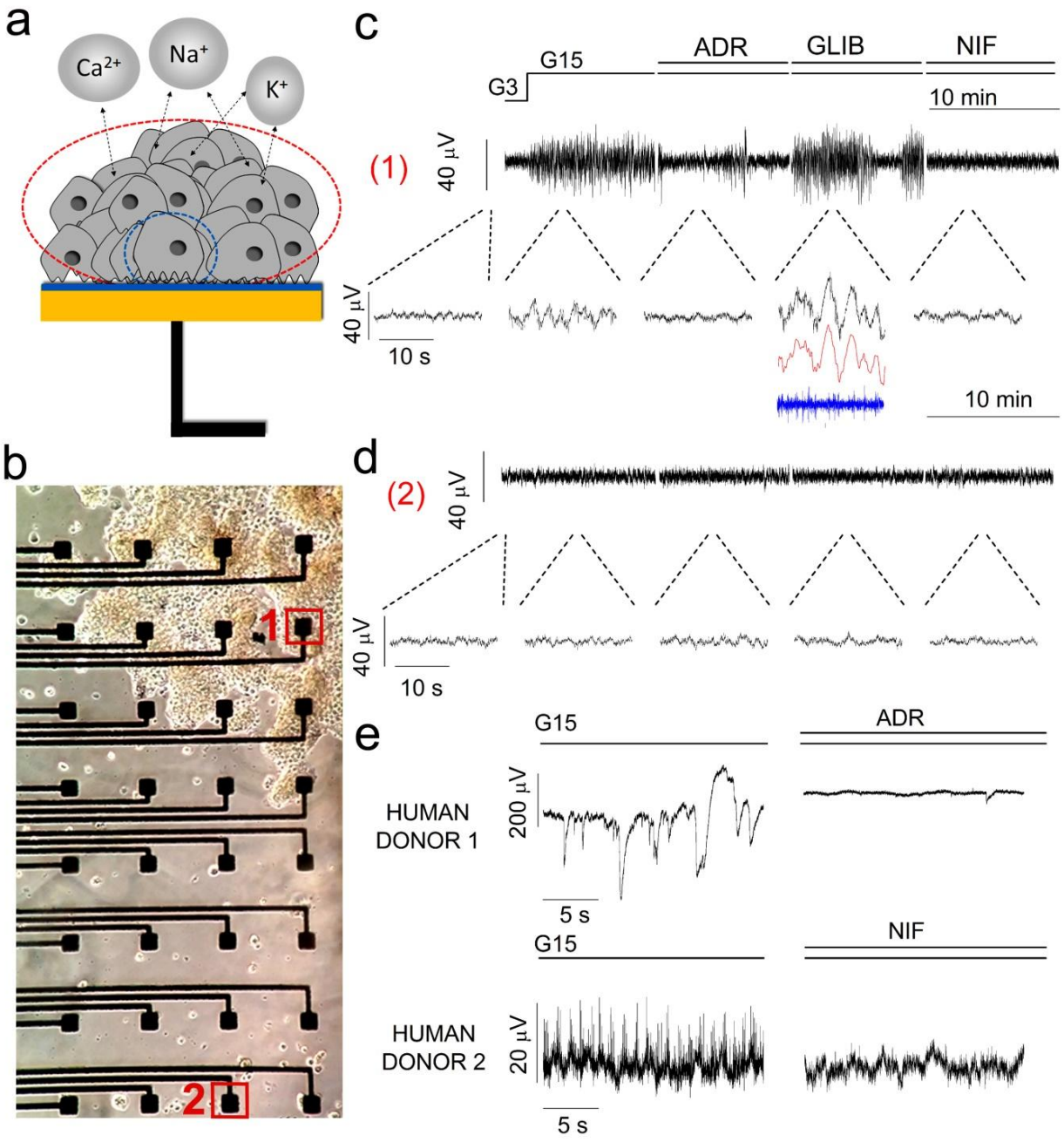


Fig. 2 Recording slow potentials and action potentials of mouse or human islets with PEDOT:PSS covered gold electrodes

a Pictorial representation of the setup with electrodes and cells. Whereas SPs reflect coupled changes of several or all β-cells (red dotted line), APs reflect the electrical activity of single cells (blue dotted line). **b** Electrodes covered by islets (1) or uncovered (2). **c** Recording of an electrode with mouse islets attached (electrode 1) exposed to low glucose (3 mM, G3) or high glucose (15 mM, G15) to which was added sequentially either the stress hormone adrenaline (ADR, 5 µM), the K_{ATP} channel closing drug glibenclamide (GLIB, 100 nM) or nifedipine (NIF, 25 µM), an antagonist of voltage-dependent calcium-channels. Magnifications demonstrate the presence of action potentials superposed on slow

potentials. The case of glibenclamide recordings after filtering are shown (black, original recordings; red, 2 Hz low pass filter; blue, 2-700 Hz band pass filter). **d** Recording from an electrode without islets. **e** Recordings from human islets obtained from two different donors (upper vs. lower panel) which were stimulated with 15 mM glucose (G15) and to which either adrenaline or nifedipine was added.

Figure 3

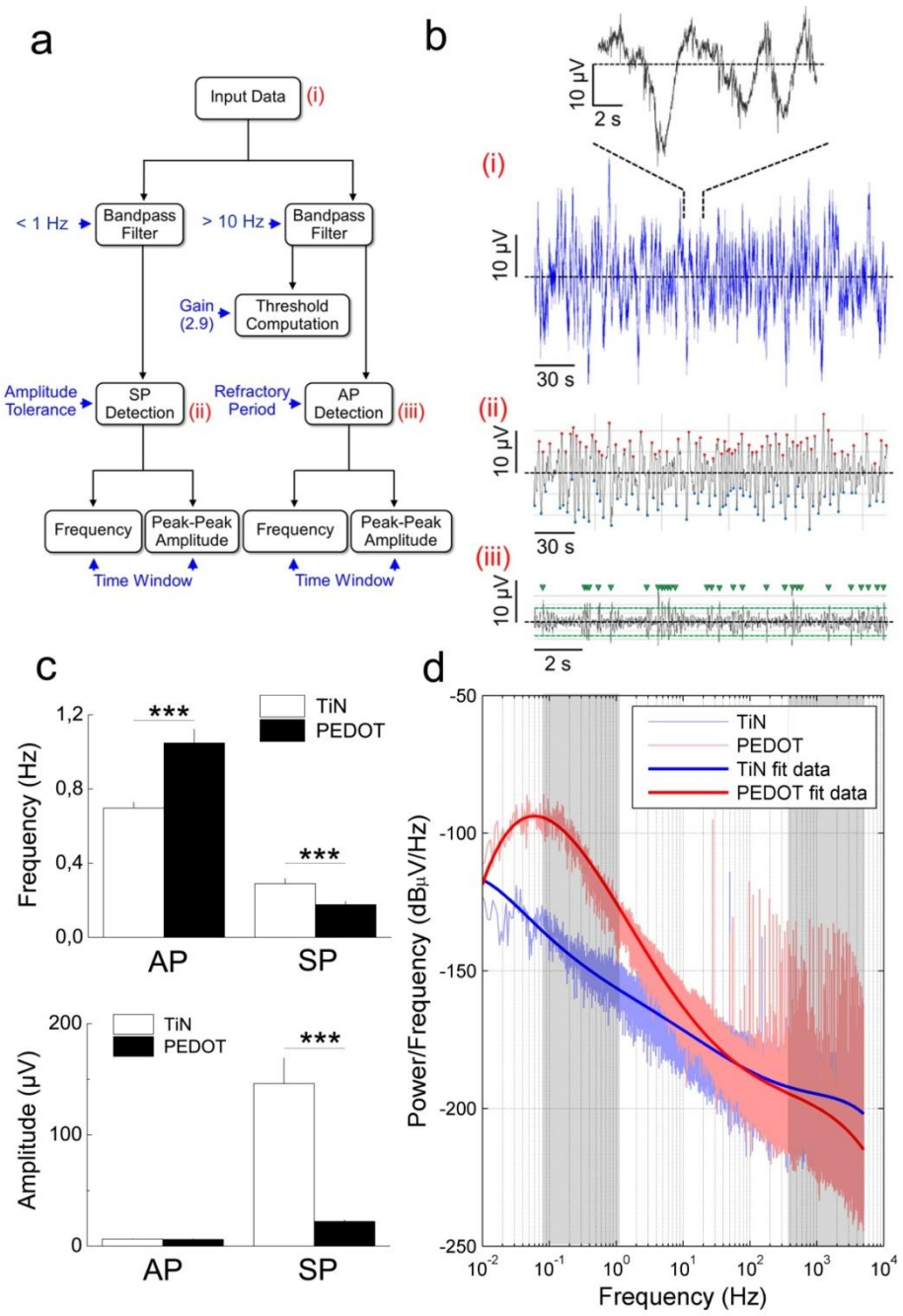
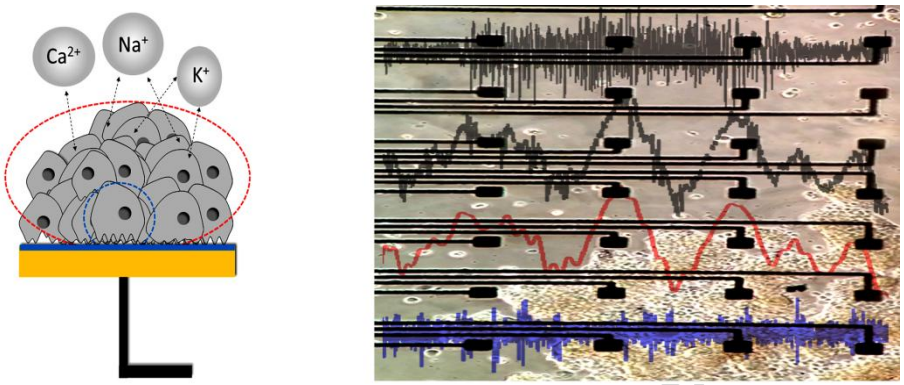


Fig. 3 PEDOT:PSS improves recording of high frequency action potentials

a Flow chart of the MATLAB routine to detect slow or action potentials. Raw input data (i) were submitted to two bandpass filters, respectively isolating APs and SPs. The AP detection chain (iii) included an adaptive threshold with a configurable gain for adjustment, and an indicative refractory period, or blanking time, which prevented multiple detections of single events. SP detection (ii) was performed according to a peak-peak amplitude tolerance, defining the minimal amplitude of a SP. For both signals, amplitude and frequency were computed in configurable sliding windows. **b** Example for representative recordings of mouse islets at 15 mM glucose on PEDOT:PSS covered gold electrodes. i,

raw data; ii, slow potentials and amplitude were measured between minima (blue dots) and maxima (red dots); iii, action potentials, thresholds are given as green dotted line, events treated as APs are indicated by inverted green triangles. **c** Comparison of amplitude and frequency for APs and SPs on TiN electrodes (open bars) or PEDOT:PSS covered gold electrodes (full bars). N= 59-61 TiN electrodes or 14 PEDOT:PSS electrodes; ***, $2p<0.005$ (Student t-test). **d** Spectral analysis of noise on TiN (blue) or PEDOT:PSS covered gold electrodes (red), which were not covered by islets, during routine recording. Given are raw data and for better comparison fitted data. The frequency range of AP and SP are highlighted by a clear grey background. Note the noise present at 50Hz and its harmonics.



Graphical abstract

Highlights

- PEDOT:PSS exhibits frequency dependent impedance
- This allows concomitant single cell action potential and micro-organ signal detection
- Single cell and micro-organ signals are automatically diagnosed and analyzed.