Polymer-based microelectrode arrays in saline and Leibovitz media

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SUMMARY: We have investigated viability and stability of platinized microelectrodes based on polyaniline (PANI). The main purpose of this work is to increase long-term stability of microelectrode arrays for field potential acquisition in neuronal cell cultures. In order to achieve a higher performance on electrode impedance, our approach has been aimed at investigating alternative conductive materials and coatings.

Conductive polymers have been lately explored on applications such as biological analysis and microelectronic devices [1,2]. In particular, biological experiments which deal with video microscopy and electrophysiological measurements may take advantage of such polymers if they may be applied onto transparent substrates and present impedance characteristics which are compatible with such systems.

Extracellular signals may be detected by means of microelectrodes, as shown for example by Oka and colleagues in [3]. Such microelectrodes may be geometrically defined on planar structures and usually present superficial areas varying from 10 to 600μm^2 [4]. Field potentials and extracellular action potentials may be then acquired in the case that their ohmic impedance for AC signals is sufficiently small (of the order of 100kΩ or less). Besides impedance characterization, mechanical stability is required in such structures, because in the case of biological cellular systems the experiment happens in a wet environment, usually immersed in physiological solution (pH 7.4). These contour conditions constrain the available spectrum of useful polymers.

In this work we investigate the applicability of polyaniline-coated microelectrodes for electrophysiological signal acquisition. Polyaniline (PANI) is a conductive polymer which has been proven useful for microelectronic devices when conveniently doped [2]. Based on these results and on a previous experience on depositing doped PANI [5] over metallic substrates we conjecture that its ohmic impedance may prove sufficiently stable when in physiological media, either for chronic or acute biopotential recordings.

Multimicroelectrode plates have been fabricated as previously reported [4]. Inside an active area of 1 mm^2 there are 100 independent microelectrodes (as shown in fig. 1). The conductive base is photolitographically defined either on gold over nickel-chromium or on SnO_2 films. Samples are then coated with PANI, as described in [5], and subsequently passivated. PECVD silicon oxide is used for passivating all structures (3000Å). Each electrode has a geometric area of 400 μm^2. Control samples (not coated with PANI) and polymeric samples are electroplatinized, using Kohlrausch solution (3%), with either 100 or 500mA/cm^2 current density [6]. Electrodepotition time and current are controlled by means Platinum black deposition may be then judged, as it is shown in fig. 2.

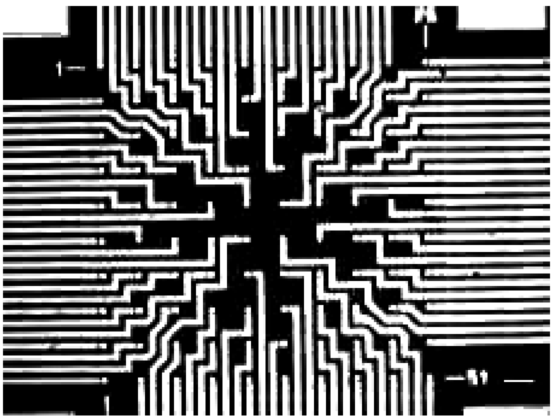

Fig. 1. Array fabricated on gold. Substrate: glass. Calibration bar (white, lower right corner): 100μm.

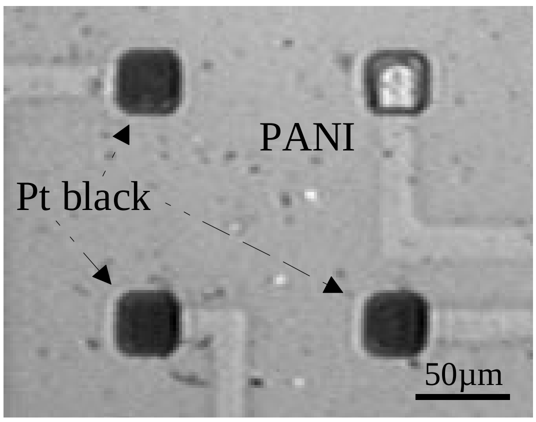

Fig. 2 - Tracks: SnO_2. Contacts: PANI and PANI coated with platinum black.

Variation in ohmic impedance is measured by means of a VI (virtual instrument) developed in LabView 5.0 (from National Instruments) running on a Pentium II connected to a DAQ16E4 (data acquisition board) and an SCXI 1100 (signal conditioning extended instrumentation), both from National Instruments. Stimuli are also generated by a VI, either through output channels of the DAQ16E4 board or by means of a GPIB-controlled Function Generator (HP33120A).

A sinusoidal signal 100mV (peak) is applied over each electrode in series with a 100kΩ resistance, and simultaneous measurement of the voltage drop over the resistance is performed. Solutions used for measuring are NaCl 0.9% (physiological saline) and L-15 (Leibovitz medium) altered for invertebrate cell culture (osmolarity 230mOsm). Unless otherwise stated, measurements have been done with a 1kHz signal.

As it can be observed by comparing figures 3 and 4, impedance characteristics do vary with time. Usually, though, PANI-coated electrodes (fig. 3) present a better stability than platinum black applied over gold (fig. 4). In this case we present 10 and 16-hour essays, where physiological saline solution has been used. Similar results are obtained when electrodes are immersed in Leibovitz medium.

Ohmic impedance is augmented by a factor of 2.42±0.06 (n=51) under L-15, independently of electrode material and passivation. Results from [7] show that with the use of a diluted MEM (minimum essential medium) it is possible to obtain the same impedance as in the case of saline solution. Although these results are apparently conflictive with ours, one has to take into consideration that L-15 and MEM have diverse composition formulas, and are used for different purposes in cell culture. L-15 (used here) is not diluted, what probably increases its impedance relatively to MEM and saline.

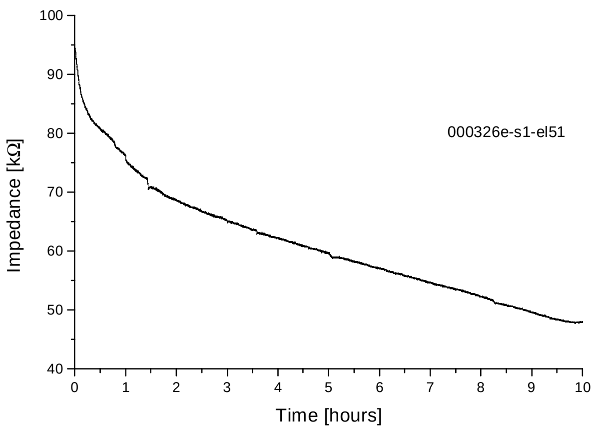

Fig. 3 - Impedance variation of a PANI coated electrode along a 10-hour monitoring period. Solution: NaCl, 0.9%.

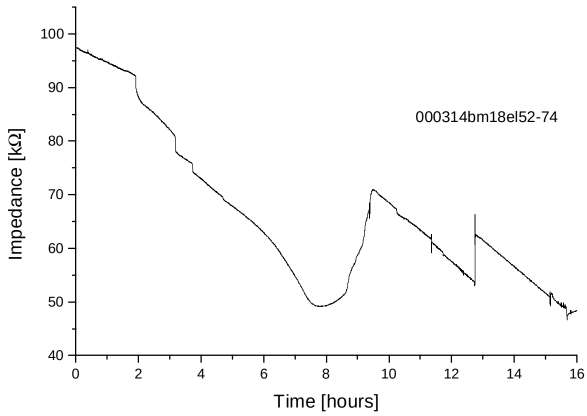

Fig. 4 - Impedance variation of a platinized gold electrode along a 16-hour monitoring period. Solution: NaCl, 0.9%.

Frequency response has been analysed and, as it would be expected from the Warburg formulas [6], we find that there is a significant increase in impedance for frequencies under 1kHz for both types of electrodes (in saline as well as in L-15 solution), and that there is a decay in impedance for higher frequencies. Figure 5 shows an example of each kind of electrode as measured in saline solution from 100Hz to 20kHz.

Mechanical stability cannot be guaranteed by PANI alone, as it may be rubbed out by cleaning and cell manipulation. Figure 6 shows four electrodes coated by PANI. In this case the polymer presents high superficial area. However, besides being only loosely attached to the golden substrate, track passivation remains an unresolved problem in this case.

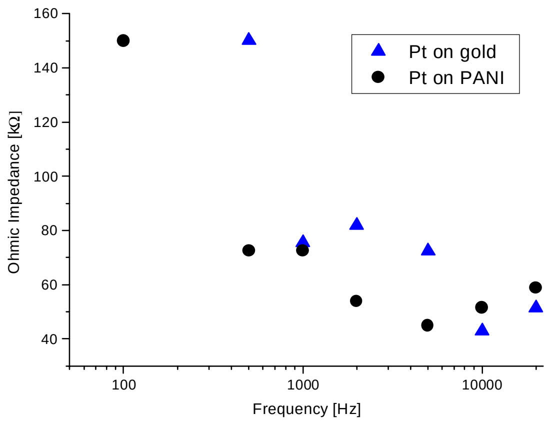

Fig. 5 - Frequency response from 100Hz to 20kHz of a Pt black electrode on gold (in blue) and a Pt black electrode on PANI (in black).

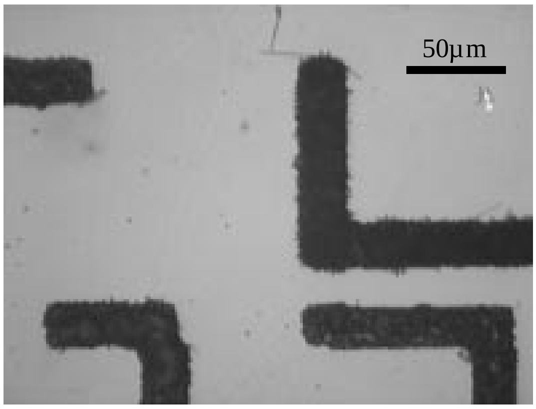

Fig. 6 - Tracks from the central area (see fig.1) coated with PANI. Calibration bar: 50µm.

Electrode resistance is monitored once a day, imersed in the electrolyte for more than an hour, in order to guarantee a stabilized ionic double-layer. Impedance is normalised to the value obtained immediately after platinization. After four days a 50% increasing is observed in the control case (in absolute values: 1.5±0.1, n=16), whereas with platinum black over PANI the rise in electrode resistance is of 33% (or 1.33±0.08, n=25).

These results show that polyaniline coating of gold electrodes may improve long term stability in the case of ohmic resistance. Moreover, although saline solution is used to monitor impedance and is considered a suitable model for *in vivo* experiments, we have shown that for recording in neural cell culture electrolytes do make a difference in the case of double layer formation, and consequently affect signal acquisition. Interface capacitance has not been monitored yet, neither the impedance behaviour when a cell is attached on the electrode.

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Acknowledgments

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