Biosensors and Bioelectronics 77 (2016) 62–68

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Single probe for real-time simultaneous monitoring of neurochemistry and direct-current electrocorticography

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ARTICLE INFO

Article history:
Received 22 August 2015
Received in revised form
9 September 2015
Accepted 10 September 2015
Available online 11 September 2015

Keywords:
Neurochemical
Direct-current electrocorticography
Spreading depolarization
Iridium oxide reference electrode
Real-time monitoring

ABSTRACT

We report a novel single neural probe for real-time simultaneous monitoring of multiple neurochemicals and direct-current electrocorticography (DC-ECoG). A major advance of this probe is the inclusion of two iridium oxide reference electrodes to improve sensor accuracy. The ECoG reference electrode is identical to the ECoG recording electrodes to significantly improve DC stability, while the reference for electrochemical sensors has 10-fold lower polarization rate to minimize the small current-induced drift in the reference electrode potential. *In vitro*, the single probe selectively measured oxygen ($r^2=0.985\pm0.01$, concentration range = 0-60 mmHg, limit of detection = 0.4 ± 0.07 mmHg) and glucose ($r^2=0.989\pm0.009$, concentration range = 0–4 mM, limit of detection = 31 ± 8 μM) in a linear fashion. The performance of the single probe was assessed in an *in vivo* needle prick model to mimic sequelae of traumatic brain injury. It successfully monitored the theoretically expected transient brain oxygen, glucose, and DC potential changes during the passage of spreading depolarization (SD) waves. We envision that the developed probe can be used to decipher the cause-effect relationships between multiple variables of brain pathophysiology with the high temporal and spatial resolutions that it provides.

1. Introduction

The brain is the most complex organ in the human body made up of a network of billions of neurons. The communications between the neurons are inherently electrical and chemical in nature through approximately 100 billion neurons with 100 trillion synapses. Hence, there is a considerable need in neuroscience research for in situ, simultaneous acquisition of the electrical and chemical signals. After acute injury to the brain, neurons at the damage epicenter depolarize, and this depression of activity spreads outward in waves through the cortex (Ayata and Lauritzen, 2015; Dreier, 2011; Leao, 1944). Decades of research show that cortical spreading depolarization (SD) can cause progressive lesion growth after ischemic, hemorrhagic, or traumatic brain injury by increasing tissue metabolic burden and diminishing perfusion (Chase, 2014; Hartings et al., 2009, 2011; Hinzman et al., 2014; Lauritzen et al., 2011; Takano et al., 2007). Detection of such processes through neuromonitoring offers the prospect of targeting patient therapies to detected intracranial pathology. However, not all pathologic brain activity is harmful, and accurate multimodal monitoring of SD and other events is needed to determine which ones require treatment. Furthermore, simultaneous electrical and chemical measures in the same brain tissue location can provide insight to cause–effect relationships among the multiple variables of interest and inform the best therapeutic approach.

Previous attempts at simultaneous electrocorticography (ECoG) and neurochemical signal recordings were based on the use of multiple, separate electrodes, which limits spatial and temporal resolution since separate sensors are not located within the same microenvironment (Dash et al., 2009; Naylor et al., 2011; Odijk et al., 2015; Rogers et al., 2013). Consequently, the development of a single probe capable of simultaneously detecting electrical and chemical signals is of great significance. Implantable micromachined microelectrode arrays represent a versatile and powerful tool to achieve this *in vivo* (Fekete, 2015; Tseng and Monbouquette, 2012; Wise et al., 2008). The techniques allow for the development of smaller electrodes that provide improved spatial and temporal resolution as well as multiple sensing electrodes with precisely defined geometries. Micro-electrochemical sensors allow

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http://dx.doi.org/10.1016/j.bios.2015.09.021
0956-5663/

the continuous, precise and fast *in situ* measurement of metabolic parameters directly within the tissue (Cordeiro et al., 2015; Hinzman et al., 2015a, 2015b; Kealy et al., 2013; Rocchitta et al., 2013; Wei et al., 2014; Weltin et al., 2014).

Recently, several neural probes have been developed for simultaneous electrophysiological and neurochemical signal recordings (Johnson et al., 2008; Morales-Villagrán et al., 2008; Song et al., 2012; Wei et al., 2015). By placing both ECoG recording electrodes and electrochemical sensors in the area of interest, near real-time monitoring of multiple parameters is possible. However, it is challenging to measure multiple variables simultaneously, especially for direct-current electrocorticography (DC-ECoG), since crosstalk between sensors introduces noise and instability. For example, DC-ECoG recording electrodes should have a very low polarization rate and stable baseline for detecting the DC shift of SD. Currently, microelectrodes for SD recording are limited to glass micropipettes with Ag/AgCl wire due to their non-polarizable property (Hinzman et al., 2015a, 2015b; Odijk et al., 2015; Rogers et al., 2013). No study to date has examined the accuracy and stability of micromachined probes for simultaneous DC-ECoG and electrochemical signal recordings.

The reference electrode is a key component for both ECoG electrodes and electrochemical sensors (Shinwari et al., 2010). However, these electrodes are not usually integrated into a single sensing probe. In previous work, an Ag/AgCl wire, screw, or platinum wire was used as a remote reference for the ECoG electrode and was placed in a comparable but inactive area such as the contralateral hemisphere, musculature, or subdermal space (Lewitus et al., 2011; Muthuswamy et al., 2005). Most electrochemical sensors used for *in vivo* studies also rely on a separate reference electrode, usually a Ag/AgCl wire (Thomas et al., 2009; Wassum and Tolosa, 2012). Ag/AgCl, however, has been shown to cause widespread necrosis in biocompatibility studies (Stensaas and Stensaas, 1978) and the implantation of multiple electrodes can cause more damage to the brain. A single probe combining all sensing and biocompatible reference electrodes can overcome this important issue, which is particularly important in the clinical setting. Such an integrated probe for *in vivo* application not only reduces tissue damage, but may also reduce noise arising from external sources and within the brain, providing improved signal-to-noise ratios (SNR) and detection limits (Li et al., 2009a; Peteu et al., 1996; Tolosa et al., 2013).

Previous studies have shown that iridium oxide (IrOx) electrodes can be a suitable replacement for Ag/AgCl wire reference electrodes for electrochemical sensors and can be used as a quasi-reference electrode for *in vivo* neuroscience applications (Tolosa et al., 2013; Yang et al., 2004). IrOx exhibits excellent biocompatibility and mechanical stability; it is stable over a range of potentials and in the presence of the ions and other components of brain extracellular fluid. Although the IrOx potential shows strong pH dependence (Ges et al., 2005), the small dynamic range of normal brain pH (7.15–7.4) makes this issue negligible (less than 30 mV potential drift) in most cases. However, IrOx has drawbacks as well. Compared to Ag/AgCl, IrOx microelectrodes have much higher impedance which not only slows the response of the potentiostats, but also increases the susceptibility of the system to environmental noise. In addition, unlike the nearly ideal non-polarization liquid–solid interface which Ag/AgCl forms in solutions, IrOx is a polarizable material, such that the passage of small currents can alter its potential. So far, the stability of the IrOx electrode potential was only studied in phosphate-buffered saline (PBS) solution; its stability as a reference electrode under continuous electrochemical sensor operation has not been reported.

In this paper, we present a new micromachined single probe for real-time simultaneous monitoring of multiple neurometabolic and DC-ECoG signals. ECoG microelectrodes are designed to be placed in gray matter to detect cortical SD, whereas electrochemical sensors are designed to be placed in white matter to measure the focal brain oxygen and glucose concentrations simultaneously. In place of separate Ag/AgCl reference electrodes, two biocompatible IrOx reference electrodes are incorporated on-probe to achieve improved DC stability and accuracy. The ECoG reference electrode is identical to the ECoG recording electrodes so that both electrodes detect the same background noise and hence increase the DC stability and SNR. The reference electrode for the electrochemical sensors has 10-fold lower polarization rate than the ECoG reference to minimize the open circuit potential drift due to small current-induced polarization. To assess the performance of the developed single probe, it was tested *in vivo* using a needle prick model that induces SD, thereby mimicking the electrophysiologic and neurochemical changes of developing brain injury. We demonstrate that this single probe can accurately monitor brain glucose, oxygen tension, and DC-ECoG simultaneously.

2. Materials and methods

2.1. Reagents and apparatus

All chemicals were analytical reagent grade or higher purity and were used as received. Chitosan (MW ~ 10^5, 75–85% deacetylation), glucose oxidase (GOD) from *Aspergillus niger* (EC 1.1.3.4), glutaraldehyde (25% in water), β-D-glucose, potassium chloride (K_2PtCl_6), sulfuric acid (99.999%), *o*-phenylenediamine (*o*PD), iridium tetrachloride hydrate, oxalic acid dehydrate (99%), hydrogen peroxide (30% solution in water), anhydrous potassium carbonate, and phosphate buffered saline (pH 7.4) were purchased from Sigma-Aldrich. All solutions were prepared with distilled water collected from Millipore Direct-Q.

All electrochemical preparations necessary for electroplating platinum nanoparticles, iridium oxide, and calibration of oxygen and glucose biosensors were performed using a potentiostat (PalmSens, Palm Instruments BV, the Netherlands). The model 215 benchtop research-grade pH/mV meter (Denver Instrument Corp., USA) was used to measure open circuit potential (OCP) of iridium oxide relative to the Ag/AgCl electrode (MF-2052; Bioanalytical Systems Inc., USA). The oxygen tensions at the difference calibration solution were measured using a precision temperature-compensated fiber-optic oxygen measurement system (OXYMICRO-Fiber-optic sensor; World Precision Instruments).

The analog outputs from both the ECoG and electrochemical sensors are sampled at 1 kHz and recorded and analyzed with a Powerlab 16/SP analog/digital converter and CHART-7 software (ADInstruments, USA). AC-ECoG signals are generated by applying band-pass filtering at 0.5–50 Hz on the original DC-coupled signals.

2.2. Probe design and fabrication

Standard microfabrication processes were used for development of the single neural probe. Briefly, the polyimide PI2611 (HD Microsystems) was spin coated at 3000 rpm for 30 s on a 4 in. silicon wafer. The polyimide was cured under nitrogen atmosphere at 450 °C for 60 min resulting in a 6 μm thick layer. After exposure to oxygen plasma (100 W, 30 mTorr) for 30 s, the metal layer (Ti/Au 120 Å/1200 Å) was deposited using e-beam metal evaporator (Temescal FC1800, BOC Edwards Temescal, USA) and patterned by photolithography to build the microelectrodes, interconnection lines and connection pads. A second PI2611 layer was then spin-coated to form a 6 μm thick layer to insulate the interconnection lines. The cured second polyimide layer was etched in oxygen plasma (200 W, 30 mTorr) for 10 min. This etching step opened