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Modification of standard CMOS technology for cell-based biosensors

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ABSTRACT

We present an electrode based on complementary metal oxide semiconductor (CMOS) technology that can be made fully biocompatible and chemically inert using a simple, low-cost and non-specialised process. Since these devices are based on ubiquitous CMOS technology, the integrated circuits can be readily developed to include appropriate amplifiers, filters and wireless subsystems, thus reducing the complexity and cost of external systems. The unprocessed CMOS aluminium electrodes are modified using anodisation and plating techniques which do not require intricate and expensive semiconductor processing equipment and can be performed on the bench-top as a clean-room environment is not required. The resulting transducers are able to detect both the fast electrical activity of neurons and the slow changes in impedance of growing and dividing cells. By using standard semiconductor fabrication techniques and well-established technologies, the approach can form the basis of cell-based biosensors and transducers for high throughput drug discovery assays, neuroprosthetics and as a basic research tool in biosciences. The technology is equally applicable to other biosensors that require noble metal or nanoporous microelectrodes.

1. Introduction

The direct interfacing of semiconductor technology to live, excitable biological tissue was initially demonstrated in the late 20th century (Fromherz et al., 1991) and has continued to be of significant interest for applications such as bidirectional stimulation and recording systems (Supplementary Information Table S1), high content screening or implantable electronic devices. The electrical interface between biological tissue and semiconductor electronics is a vital component of a successfully functioning system and often takes the form of a low impedance electrode (Graham et al., 2011a; Hierlemann et al., 2011). The electrode area of the semiconductor die must be both biocompatible and chemically inert which has led to the use of existing manufacturing methods requiring specialist and expensive device manufacture. This has limited their application in key areas such as high-throughput screening and drug discovery.

This article presents an economical, flexible and biocompatible bio-sensing device based on standard CMOS technology in which aluminium electrodes are modified using simple anodisation and plating techniques. The modification process does not require intricate and expensive semiconductor post-processing equipment and can be performed with simple bench-top equipment. A clean-room environment is *not* required. This new approach provides the basis for low-cost mass-manufacture of electrodes. We demonstrate that the modified CMOS is readily configured for neuron action potential recordings and cell-substrate impedance systems and such an approach can be used in many other applications such as DNA hybridisation (electrophoresis) (Liu et al., 2006) and DNA sequencing (Ion Torrent Systems Inc., 2010). Individual microelectrodes are addressable using on-chip circuits and can meet the requirements for long-term and non-invasive cell culture assays and label-free high content screening.

2. Materials and methods

2.1. CMOS device construction

The CMOS integrated circuits (ICs) comprised of 48 electrodes for recording extracellular action potential activity in adult mammalian neurons and for sensing the electric cell-substrate impedance (ECIS) of an epithelial cell line.

The CMOS ICs were fabricated by austriamicrosystems AG, Germany using 0.8 μm technology. Thirty devices were supplied in 48-pin ceramic dual in line (DIL) packages with removable die-cavity lids. A further 18 bare dice were partially encapsulated

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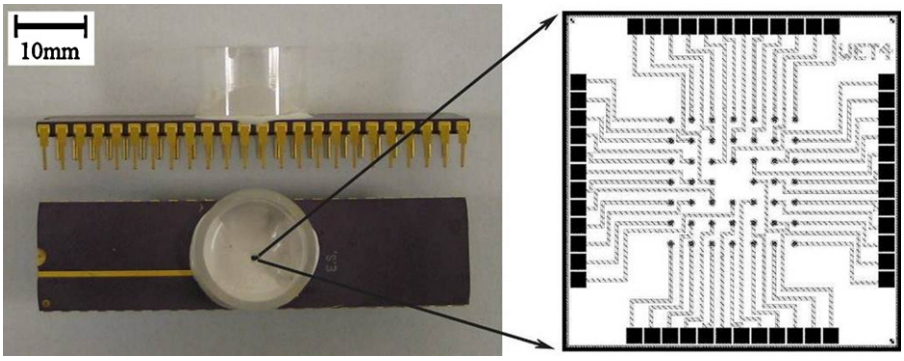

Fig. 1. The CMOS device and electrode layout. The silicon dimensions are 3.1 mm × 3.1 mm.

by Quik-Pak (San Diego, U.S.) using their standard manufacturing process, except that biocompatible compounds were specified, namely: Silastic Medical Adhesive Silicone Type A (Dow Corning, U.S.) for placement of the window frame and Hysol CB064 (Loctite, U.S.) for encapsulation (Graham et al., 2011b). A cyanoacrylate adhesive was used for a permanent bond, or the glass adhered using Silastic 9161 (Dow Corning, UK) so that the packages could be more easily disassembled, e.g. for scanning electron microscopy analysis.

Glass cylinders, 10 mm tall, 13 mm internal diameter (QB Glass, UK), were adhered to the upper surface of the packages encircling the open die cavities so that the 48 electrodes were exposed at the base of each chamber. These formed receptacles for further processing of the electrodes and subsequently functioned as cell culture chambers (Fig. 1).

To interface the CMOS technology directly with biological tissue, while maintaining biocompatibility and chemical inertness the aluminium electrodes were anodised to form nano-porous alumina (aluminium oxide). The nano-pores were subsequently infiltrated with gold and plated (Graham et al., 2009a,b, 2010) with platinum black to reduce and optimise the electrode electrical impedance for neuronal recordings (Fig. 2). This economic and simple CMOS modification process begins with anodisation of the aluminium electrodes for approximately 40 min in 0.4 M phosphoric acid, followed by 20 min plating of the anodised nano-porous alumina in 59 mM gold chloride ($HAuCl_4 3H_2O$) and approximately one minute for platinum black deposition using chloroplatinic acid ($H_2PtC1_6 6H_2O$) with 264 μM Lead(II) acetate trihydrate. Fig. 2 (supported by Supplementary Fig. S1) shows a section of an electrode processed using this method, illustrating the uniform anodisation and gold deposition across the area of the electrode. Full details of the method were presented in Graham et al. (2010). The net result of this process is that the CMOS aluminium electrode, which normally corrodes when in contact with a saline culture medium, is converted to a chemically inert, low impedance electrode consisting of aluminium oxide, gold and high surface area platinum black.

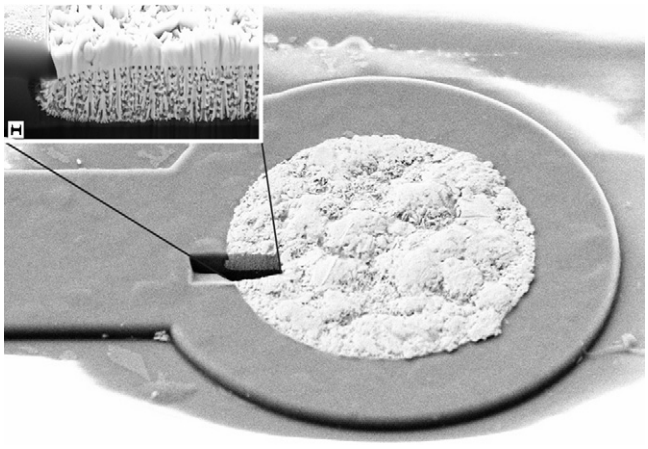

Fig. 2. Scanning electron microscope (SEM) image of a single electrode with section created by milling with a focused ion beam (FIB). The inner circular area is the electrode (30 μm diameter). This is surrounded by approximately 10 μm of aluminium which appears as an outer 'ring' which is connected to the aluminium track leading off to the left. The inset shows the porous alumina filled with gold. Inset scale bar 200 nm.

2.2. Cell culture

Rat dorsal root ganglion cells were cultured as previously described (England et al., 2001). In brief, adult (250 g) Sprague-Dawley rats were housed and killed according to UK Home Office regulations. The dorsal root ganglia were dissected into Ham's F14 media supplemented with penicillin (100 IU ml^{-1} Sigma, UK), streptomycin (100 μg ml^{-1}, Sigma) L-glutamine (2 mM, Sigma) and 10% foetal calf serum (FCS, Invitrogen, UK). The ganglia were transferred to F14 medium containing 0.125% collagenase and incubated at 37 °C in 5% CO_2 95% air for 40 min. The cells were further transferred into F14 medium with added nerve growth factor (50 ng ml^{-1}) and plated on the sterile CMOS devices which had been previously coated with poly-L-lysine (15–30 kDa, 0.01%, w/v). The devices were then kept at 37 °C in 5% CO_2/95% air for 18–24 h.

The Caco-2 human epithelial cell line was chosen for the ECIS recordings as the cells continually divide to produce a monolayer (Hidalgo et al., 1989). Cells were cultured in DMEM/F-12 media supplemented with L-glutamine (1%, w/v, Sigma), FCS (10%, w/v) and non-essential amino acids (1%, w/v). Cells were grown in 50 ml flasks (Nunc, UK) to 90–100% confluency at 5% CO_2 and 37 °C. The cells were dissociated from the flasks by 3–6 min trypsinsation (0.5%, w/v, Worthington) after having been rinsed in EDTA (10 mM, Sigma, UK) and calcium and magnesium free phosphate buffered saline. Detached cells were spun down (5 min at 700 rpm), re-suspended in fresh medium and plated onto the sterilised (ethanol 30 min) devices.

2.3. Electrophysiological recording

To demonstrate that the simply modified CMOS can sense the fast electrical activity of neurons, primary cultures of rat dorsal root ganglion (DRG) cells (England et al., 2001) were plated onto poly-lysine coated CMOS devices that were incubated for 18 h, as described in the previous section. Recordings were undertaken at 21 °C by placing the CMOS devices in a 48-pin zero insertion