PHD

Biocompatible Low-Cost CMOS Electrodes for Neuronal Interfaces, Cell Impedance and Other Biosensors

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Biocompatible Low-Cost CMOS Electrodes for Neuronal Interfaces, Cell Impedance and Other Biosensors

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A thesis submitted for the degree of Doctor of Philosophy

University of Bath
Department of Electronic & Electrical Engineering
June 2010

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.........................................................
Abstract

The adaptation of standard integrated circuit (IC) technology for biosensors in drug discovery pharmacology, neural interface systems, environmental sensors and electrophysiology requires electrodes to be electrochemically stable, biocompatible and affordable. Unfortunately, the ubiquitous IC technology, complementary metal oxide semiconductor (CMOS), does not meet the first of these requirements. For devices intended only for research, modification of CMOS by post-processing using cleanroom facilities has been achieved by others. However, to enable adoption of CMOS as a basis for commercial biosensors, the economies of scale of CMOS fabrication must be maintained by using only low-cost post-processing techniques. The scope of this work was to develop post-processing methods that meet the electrochemical and biocompatibility requirements but within the low-cost constraint. Several approaches were appraised with the two most promising designs taken forward for further investigation. Firstly, a process was developed whereby the corrodible aluminium is anodised to form nanoporous alumina and further processed to optimise its impedance. A second design included a noble metal in the alumina pores to enhance further the electrical characteristics of the electrode.

Experiments demonstrated for the first time the ability to anodise CMOS metallisation to form the desired electrodes. Tests showed the electrode addressed the problems of corrosion and presented a surface that was biocompatible with the NG108-15 neuronal cell line. Difficulties in assessing the influence of alumina porosity led to the development of a novel cell adhesion assay that showed for the first time neuronal cells adhere preferentially to large pores rather than small pores or planar aluminium. It was also demonstrated that porosity can be manipulated at room temperature by modifying the anodising electrolyte with polyethylene glycol.

CMOS ICs were designed as multiple electrode arrays and optimised for neuronal recordings. This utilised the design incorporating a noble metal deposited into the porous alumina. Deposition of platinum was only partially successful, with better results using gold. This provided an electrode surface suitable for electric cell-substrate impedance sensors (ECIS) and many other sensor applications. Further processing deposited platinum black to improve signal-to-noise ratio for neuronal recordings. The developed processes require no specialised
semiconductor fabrication equipment and can process CMOS ICs on laboratory or factory bench tops in less than one hour.

During the course of electrode development, new methods for biosensor packaging were assessed: firstly, a biocompatible polyethylene glycol mould process was developed for improved prototype assembly. Secondly, a commercial ‘partial encapsulation’ process (Quik-Pak, U.S.) was assessed for biocompatibility. Cell vitality tests showed both methods were biocompatible and therefore suitable for use in cell-based biosensors.

The post-processed CMOS electrode arrays were demonstrated by successfully recording neuronal cell electrical activity (action potentials) and by ECIS with a human epithelial cell line (Caco2).

It is evident that these developments may provide a missing link that can enable commercialisation of CMOS biosensors. Further work is being planned to demonstrate the technology in context for specific markets.
Acknowledgements

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List of Common Abbreviations

a.c. alternating current
CAM cell adhesion molecule
CMOS complementary metal oxide semiconductor
CPA chloroplatinic acid
CPE constant phase element
d.c. direct current
DEP dielectrophoresis
DIL dual in-line
DIP dual in-line package
DIV days in-vitro
DNA deoxyribonucleic acid
DRG dorsal root ganglion
ECIS electric cell-substrate impedance sensor
ECM extracellular matrix
EIS electrochemical impedance spectroscopy
EOS FET electrolyte oxide semiconductor field effect transistor
expt experiment
FES functional electrical stimulation
FET field effect transistor
FIB focussed ion beam
HCS high content screening
HTS high throughput screening
IC integrated circuit
ILD inter-layer dielectric
IMD implantable medical device
ISFET ion sensitive field effect transistor
KCL King’s College London
MCS Multi Channel Systems
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>MEA</td>
<td>multiple electrode array</td>
</tr>
<tr>
<td>MEMS</td>
<td>micro electromechanical system</td>
</tr>
<tr>
<td>MOS</td>
<td>metal oxide semiconductor</td>
</tr>
<tr>
<td>n-DEP</td>
<td>negative dielectrophoresis</td>
</tr>
<tr>
<td>OCP</td>
<td>open circuit potential</td>
</tr>
<tr>
<td>PED</td>
<td>pulsed electrodeposition</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
</tr>
<tr>
<td>PLL</td>
<td>poly-l-lysine</td>
</tr>
<tr>
<td>pSi</td>
<td>porous silicon</td>
</tr>
<tr>
<td>r.m.s</td>
<td>root mean square</td>
</tr>
<tr>
<td>RTV</td>
<td>room temperature vulcanisation</td>
</tr>
<tr>
<td>SCE</td>
<td>saturated calomel electrode</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscope</td>
</tr>
<tr>
<td>TEM</td>
<td>tunnelling electron microscope</td>
</tr>
<tr>
<td>TFT</td>
<td>thin film transistor</td>
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**Organisation of the Text**

A broad view of the literature is taken in the Introduction (Chapter 1). This sets the background for a more focussed review in Chapter 2, leading to a description of CMOS biosensors used for extracellular neuronal recordings in Section 2.9.

Methods and technologies are presented only as required since they would have no context if discussed in Chapters 1 or 2. For example, literature relating to barrier oxide thinning is only introduced when called upon by Section 3.2.2.1. It is hoped this approach makes it easier to place the technical concepts into context. The reader will probably find a plethora of technical terms from outside their field. Effort has been made to explain these terms through the introductory chapters, but it is hoped that sufficient referencing will also help the reader in this respect.

The thesis is developed through Chapter 3 but also introduces specific concepts such as electroplating to ensure the premise of the work is sound before embarking on experimental investigations. Chapter 4 is a brief section to assist the reader in navigating the subsequent experimental work of Chapters 5 to 11.

A ‘key points’ box is included at the end of each chapter to highlight the important features of the work. Novel aspects are highlighted in red and other key points are summarised in blue.
1 Introduction

Biosensors are a cornerstone of neuroscience. Helping the paralysed to walk, improved food safety, an artificial human eye, new approaches to drug discovery, prosthetic and performance-enhancing brain implants, defence against biological warfare, affordable research tools for neuroscience. These are just a few of the varied applications of biosensors and are the inspiration and motivation for this work. However, no single science discipline can turn what remains primarily science fiction into reality. Instead, progress and successes are only being made through a highly multi-disciplinary approach. A single application may require leading edge contributions from neuroscientists, biologists, semiconductor engineers, electronic hardware designers, pharmacologists and surgeons.

However, as an alternative to this socio-economic viewpoint, one can take a bottom-up approach starting with the generic observation that today’s world of computerised systems comprises devices which all have an input and an output. Input from the real world requires some form of interface, whether it is a computer peripheral to detect the press of a key, a thermocouple to measure the temperature inside a combustion engine, or a conductive pad that detects the electrical activity of a heart. These are all sensors in so much as they create a signal, in some form or another, related to the characteristics they are designed to measure or an event they are intended to detect. A biological sensor or ‘biosensor’ is therefore a device that measures or detects a biological-related characteristic.

Breakthroughs come about by the marriage of biosensor technologies to each specific application. There are often common requirements to biosensors such as a need for compatibility with their biological surroundings – biocompatibility. However, the field is so wide yet the requirements so specific that each application often has many requirements that are unique, such as gaining an improved compatibility between long-term (chronic) brain implants and surrounding tissue by building the electronics on flexible materials [1]. Indeed, using biosensors to unite the nervous system with the computing and signal processing abilities of electronics is probably the most complex and ambitious of applications yet the rewards of this pursuit is the primary stimulus for this work.
1.1 An Overview of Biosensors

One of the first publications to define and discuss biosensors was ‘Biomedical Telemetry’ in 1969 [2]. At this time, integrated circuits were in their infancy and a patent for the cornerstone of modern electronics – CMOS – had only recently been granted [3]. The meaning of the term ‘biosensor’ now has a well-defined core but, like many technological fields, it still has vague edges. In 1992, the International Union of Pure and Applied Chemistry adopted the concise definition of biosensors as [4],

‘A device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals.’

This definition has been open to debate: some would state that biosensors include systems that measure characteristics related to biological activity, such as concentration, but which do not include biological elements as part of the sensor. A chemical sensor that directly detects a gaseous biohazard is such an example. Sensors that do include a biological component may appear in many guises. These biosensors can be a whole organism [5], an individual sensing organ taken from a primitive organism [6], individual but whole cells [7], or biological components (molecules) such as antibodies, nucleic acids and enzymes [8]. So the introduction of new technologies has put pressure on the terminology to keep pace, as demonstrated in the much fuller definition currently provided by the journal ‘Biosensors and Bioelectronics’ [9],

‘Biosensors are defined as analytical devices incorporating a biological material (e.g. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, natural products etc.), a biologically derived material (e.g. recombinant antibodies, engineered proteins, aptamers etc) or a biomimic (e.g. synthetic receptors, biomimetic catalysts, combinatorial ligands, imprinted polymers etc) intimately associated with or integrated within a physicochemical transducer or transducing microsystem, which may be optical, electrochemical, thermometric, piezoelectric, magnetic or micromechanical.’

This difficulty with terminology comes as no surprise since the past two decades have seen a rapid growth in biosensor design and technological approaches (Figure 1). So whilst a full
description of all application areas and their underlying technologies becomes prone to omission, the following sections (1.2 and 1.3) serve to illustrate the core areas of research and development in the field of biosensors.

Figure 1. Biosensor publications: academic articles and patents (Data source: ISI Web of Knowledge [10])

1.2 Biosensor Applications and Markets

Biosensors have been developed during the past thirty years, but have only recently become commercially viable. For true biosensors (i.e. excluding larger scale systems that are sometimes now classed as biosensors) the world market in 2001 was $1.2 billion [11]. Many emerging biosensor applications take the approach of ‘lab on a chip’ which typically includes a micro electromechanical system (MEMS).

Medical biosensors may be a component of diagnostic instrumentation, instantaneous biological parameter measurement, or monitoring devices. Monitoring devices may also contain actuators for the administration of drugs or control of biological parameters – a most successful example of which is the cardiac pacemaker. Despite the established use of pacemakers to control the rhythm of heart muscle (cardiomyocytes), the interface between electronics and excitable cells – mainly neuronal cells and myocytes – is at an embryonic
stage of development. However, the goal is that development of this technology will assist in the diagnosis, management and restoration of nervous system disorders [12].

1.2.1 Medical Implants
Since the foundation of implant technology, many forms of electrode for neuronal stimulation and recording have been used, but the need for chronic interfacing and increased information transfer have driven developments such as biocompatible electrode materials and multiple electrode arrays (MEAs).

Relatively simple electrodes have been used in commercially successful implantable medical devices (IMDs) for functional electrical stimulation (FES) neuroprosthetics such as the pacemaker, ‘Dropped Foot’ stimulator [13], sacral nerve stimulation for bladder control (e.g. Interstim [14]), cochlear implants (e.g. from Med-El [15] and Advanced Bionics [16]) and paralysed muscle reanimation (e.g. the BION® from the Alfred Mann Institute [17]).

The classic and more ambitious goal of restoring function after neurological-based paralysis is still very much work in progress: the relation between individual neurons in the brain and specific limb movement has been investigated for over twenty years [18]. During that period there have been significant advances in the neuron-electronic interface and supporting technologies such as wireless, but the achieved movement remains poor and unnatural [19]. In fact the remaining barriers to achieving movement initiated by a cortical (brain) implant are still so significant that some experts still question whether it will ever become reality [20].

1.2.2 Pharmacology and Drug Discovery
Before the advent of modern medicine, man used a hit-and-miss method of finding therapeutics, usually herbal remedies [21]. This trial and error process has generally moved from the population as a whole to multi-national pharmaceutical companies yet the methodology has not really changed: the front-end of the discovery process remains a test of thousands or even millions of potential compounds and looks for ‘hits’. This process is termed ‘high throughput screening’ (HTS). However, a significant difference between the traditional method and modern HTS is that since the 1980’s the structure under test usually consists of molecular targets rather than the whole body: the targets are either chemical or biological but
most commonly proteins and occasionally whole cells [21]. The target is then tested against a selection of typically $10^5$ to $10^6$ compounds from the library of up to $10^7$. Due to the large number of tests, microwell plates are used, each plate commonly having 384 wells, but plates with up to 1536 wells are readily available [22]. Possibly hundreds of ‘hits’ from the screen may be detected, but many are often spurious results which need confirmation, perhaps with ten confirmed hits. Further analyses of the data gives ‘leads’. The process continues through lead optimisation to a drug candidate. This established process is chiefly dependent on attaching radioactive, enzyme or fluorescent labels to the targets [23]. These labels then signal a response such as a binding of a ligand (i.e. a library compound) to its molecular target (e.g. receptor or binding site on a target protein or cell). However, the preparation of such labelled targets is time-consuming, costly and may interfere with the very interactions that are under test [24]. More recently, measurements have used more in-depth analysis of the compound responses through a deeper analysis of biological effects during the test. This ‘high content screening’ (HCS) in drug discovery may, for example, be achieved through confocal microscopy – enabling measurement of individual cells [25]. HCS products are generally available through large corporations such as GE Healthcare (IN Cell Analyzer [26]), TTP LabTech (Acumen [27]), Thermo Fisher Scientific Cellular Imaging (ArrayScan and cellWorx [28]) and LemnaTec (Scanalyzer [29]). However, these methods still most commonly use labelled cells with all the above mentioned drawbacks.

To overcome the problems of labelling, ‘label-free’ assays have generated increasing interest within the HTS market. An approach that can dispense with the labelling is to use biosensors to detect chemical or physiological changes (hits). A continually increasing number of label-free biosensors are now able for the HTS market [24]. Several of these solutions still use optically-based detection (e.g. resonant waveguide grating [30], surface plasmon resonance (SPR) [31]), but several also include cell-based biosensors, such as electric cell-substrate impedance sensing (See Section 2.6) [32]. The use of cell-based assays in ECIS has the advantage that hits are more likely to be true physiological reactions (i.e. fewer false positives), but one limitation of the technology is caused by the multiple electrical connections required to each well of the multiwell plate [33],[34].

Ion-channel assays are available that detect electrophysiological responses, which are particularly useful for development of cardiovascular and nervous system drugs. There are already several commercial ion-channel HTS products and these generally use either labelled
cells [35],[36] or automated patch clamping\textsuperscript{1} (e.g. IonWorks, Molecular Devices Inc. [37],[38]). The latter require proprietary substrates, such as those with suction holes to form a patch clamp. These approaches appear to dominate the market at present with no truly high throughput solutions using planar extracellular electrodes. A reliable low-cost sensor design therefore has the scope for significant penetration into the HTS market. The cost of individual electrodes is important since they are likely to be used in large quantities, such as one in each well of a 384 multiwell plate. However, success will only come after significant difficulties related to cell culture and adhesion have been overcome [38].

\textbf{1.2.3 Industrial and Defence Applications}

Beyond implants and drug discovery, there lies a broad spectrum of other applications for biosensors in medicine. A key breakthrough was seen with the introduction of glucose monitors for diabetics in the 1970’s leading to the commercial strips and pocket-sized monitors available today. Real-time \textit{in-vivo} monitoring is a present focus of research and requires small coated sensors with appropriate power sources [39]. Such enzyme-based sensors extend to, for example, urea, alcohol, immunosensors and medical microbial biosensors. Further markets for biosensors lie in environmental applications such as the detection and monitoring of pollutants. Detection of bacteria and toxins are applications for both food safety and as defence against biological warfare [40],[41] or in agriculture in the form of pesticide, organophosphate residue detection [42] and seed vitality assessment [43].

Traditional methods of pathogen detection in food such as culturing of cells and colony counting are slow, typically taking hours or weeks before results are obtained [44]. Biosensors have the promise of much faster measurements and in-situ analysis, but further research is still required to improve both sensitivity and selectivity [45].

\textsuperscript{1} The patch clamp is a standard technique in electrophysiology for measuring and influencing the intracellular potential or currents of cells and for studying ion channels. See Figure 15 (page 33) for further details.
1.3 Biosensor Technologies

An ever-increasing portfolio of biosensor technologies exists and these can be classified as illustrated in Figure 2 [45],[46]. A functional sensor comprises two parts: firstly, a biological receptor or ‘bioreceptor’ that detects the presence of the substance under test (the analyte) and, secondly, a transducer that detects a response of the bioreceptor which then translates this into an output signal. The sensing bioreceptor is usually immobilised on the chemical/physical transducer either by natural adhesion processes or by coating the surface (Figure 3) [6].

Figure 2. Classification of Biosensors (Adapted from [45]).
Figure 3. Elements of a biosensor. Various biological elements may form the bioreceptor which are immobilised on the transducer. (Adapted from [47].)

Figure 4. Analysis of the literature containing the terms ‘CMOS’ or ‘integrated circuit’, organised by the bioreceptor classifications of Figure 2 (Data source: ISI Web of Knowledge and Google Scholar, 3rd Feb 2010).
1.3.1 Bioreceptors

Bioreceptors are those sensors that use a biochemical mechanism as the detector and most commonly include cells, DNA, enzymes and antibodies [48]. This is reflected by an analysis of the literature (Figure 4) which discusses these bioreceptors in relation to integrated circuits. Antibodies and antigens are immune system proteins that can be bound to a transducer are particularly useful in the development and recognition of antibodies (immunosensors) [49]. The antibodies are most frequently labelled to allow detection and enzymes are commonly implemented as the label. A biosensor substrate can be coated with DNA (deoxyribonucleic acid) which can then detect the unique sequence of corresponding base pairs [50],[51] and has found many applications in the detection of food pathogens [45]. The complexity of cell-based biosensors enables them to respond to a large range of stimuli and, because of the inherent direct measurement, they are also capable of responding to previously unknown agents. The whole-cell response is effectively the primary transducer and therefore requires a secondary transducer to detect the primary response [8]. Optical methods can be used as the secondary transducer in cell-based biosensors, as performed in [52] where microscopy was used to count the number of viable cells in multiwell plates after exposure to food-borne pathogens. A benefit of cell-based detectors is that they are able to distinguish between viable (live) and non-viable analytes (e.g. pathogens). Expressed more generally, cell-based biosensors can directly detect physiological changes, e.g. to toxins, and so can provide detection of unknown or unexpected ‘agents’, whereas molecular biosensors will often detect only specific agents [53]. Additional bioreceptors include ‘biomimetic’ components – which are synthesised to mimic antibodies, enzymes, DNA or cells – and bacteriophages (‘phages’) which are viruses that bind to, and can therefore identify, specific bacterial pathogens [54].

1.3.2 Transducers

Transducers can broadly be classified into three groups, based on optical, electrochemical and mass-based detection methods. Of most interest here are the electrochemical techniques that are more relevant to IC-based biosensors. Amperometric transduction [45] is a current-measuring biosensor of two electrodes, operated at constant potential and is highly sensitive to the concentrate of an analyte [55]. Conductometric transducers operate in a similar manner by detecting changes in electrical conductance of an analyte. Similarly, potentiometric detection comprises a measure of potential at zero current (known as the ‘open circuit potential’ or OCP) and varies logarithmically thereby allowing detection of very small
changes in analyte concentration. However, it may be noted that the distinction between amperometric, conductometric and potentiometric methods is largely historical and has become less important due to the increasing flexibility of modern instrumentation [56]. An extension of these methods is the light-addressable potentiometric sensor (LAPS) which detects changes in the junction potential of a doped silicon layer of when subjected to a photocurrent produced from an external light source [57]. An impedimetric (impedance) transducer is another form of electrochemical sensor that can be used in the label-free measurement of viable cells [34]. These cell-substrate impedance sensors (ECIS) are discussed to greater depth in Section 2.6. Piezoelectric materials can be used as mass transducers by detecting the additional mass of chemicals binding to the surface, for example coated with an antibody or other bioreceptor [58]. The frequency of piezoelectric crystal oscillation varies with applied mass which can then be detected electrically (QCM², SAW³). Lastly, biosensor transducers using the magnetoelastic properties of ferromagnetic materials may also be used to detect changes in mass when they are used as resonating micro-cantilevers [59].

1.3.3 Transducer Suitability for CMOS Manufacture

Constructing an overall picture of biosensor research is impeded by the large quantity of published articles and patents combined with the diversity of the technologies and applications. However, a quantitative analysis of the literature (Figure 5) shows the most commonly reported integrated circuit biosensors use cell-based bioreceptors with optical fibre or piezoelectric transducers. The fibre optic element is not a true transducer in its own right since its role is more accurately a conduit to a transducer that is remote from the bioreceptor and analyte [60]: these remote sensors can use various spectroscopic techniques such as absorption, fluorescence, phosphorescence, surface plasmon resonance. Additionally, cell-based biosensors using piezoelectric, magnetoelastic or optical-based transducers (Raman, FTIR⁴, SPR⁵) are not readily implemented in standard CMOS integrated circuits and therefore lie beyond the scope of this work. Conversely, electrochemical (amperometric, potentiometric, conductometric, impedimetric) transducers are most suited to manufacture using standard CMOS processes since electrodes in contact with an analyte can be readily formed on the surface of the integrated circuit (The formation of CMOS electrodes is

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² QCM – Quartz Crystal Microbalance
³ SAW – Surface Acoustic Wave
⁴ FTIR – Fourier Transform Infrared Spectroscopy
⁵ SPR – Surface Plasmon Resonance
discussed in Section 1.4). These electrodes may be used in conjunction with the various types of bioreceptor discussed above and are reviewed in Section 2.9.

Figure 5. Further analysis of the dataset in Figure 4 sub-divided by the transducer classifications of Figure 2. The results are for indication only since some of these articles returned against the search terms will be irrelevant.

The use of complex IC technology in a biosensor application naturally needs justification. Where, for example, a passive device is called for, requiring no transistors and only a single layer defines tracks and electrodes, then this may often be cost-effectively produced using a custom manufacturing process using simple photolithographic methods. However, it is when a specification calls for circuitry close to the electrodes, such as low noise pre-amplifiers for neuronal recordings, that the benefits of using CMOS are obviously realised. Research biosensors using CMOS presently access mature fabrication processes, with the ability to define features only 0.1 µm or larger. However, the industry is currently working toward features as small as 22 nm for 2011 [61]. The economies of scale resulting from volume manufacturing and the ability to pack data processing capabilities into very small areas of
silicon chip will enable excellent spatial resolution of biosensor electrodes and a much lower device cost than can be achieved using a custom manufacturing process.

1.4 The Electronic-Neuronal Interface

Passive MEAs have generally had limited spatial resolution and are expensive to manufacture. As a result, much work begun during the 1990’s in order to leverage the potential benefits of planar semiconductor technologies – primarily CMOS – offering a potentially cheap source of electrodes, integrated signal processing and increasingly excellent spatial resolutions. The cost-effectiveness of using CMOS ICs comes from restricting the construction of electrodes to the materials present in standard bondpads – mostly aluminium and alloys thereof. This is the basis of the approach taken in this thesis. Unfortunately, aluminium is stated in the literature as a known neurotoxicant, although little supporting information is given [62],[63].

Early investigations, especially by groups such as The Max Planck Institute of Biochemistry (Fromherz et al.), identified the adherence of biological cells to the IC electrodes as a major challenge [64]. Progress has been slow as the factors influencing cell-substratum adhesion are complex. These themes are explored to greater depth in Sections 2.5 and 2.9.

1.5 Packaging Technology

A biosensor generally requires some form of carrier or package to support and position the active sensor. However, a key requirement of most biosensors is that the active sensor area is exposed to the external environment it is to measure. This is different from most other forms of IC where input is through the package external electrical connections (e.g. leads or solder bumps) and these form a seal between the internal device and the environment. IC sensors may, for example, measure temperature, pressure, acceleration or light intensity (e.g. a photodiode or camera array) but these ICs can usually be sealed from their external environment (e.g. using a transparent window for light sensors) [65]. The micro-

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6 Passive MEAs are generally limited by pin-out. For example, the Multi Channel Systems MEA range is limited to 60 electrodes in a 19 mm diameter culture chamber – a resolution of $2 \times 10^5 \text{m}^{-2}$. The cost of these MEAs is approximately £350–400.

7 With on-chip signal multiplexing and processing, large CMOS arrays may be formed with spatial resolution limited by the cell culture density rather than the IC technology. For example, a 128 x 128 array of 10 µm diameter electrodes at 50 µm spacing would be a resolution of $4 \times 10^8 \text{m}^{-2}$. 

12
electromechanical systems (MEMS) market initially leveraged the semiconductor industry for both substrate and packaging technology, but the specific requirements of MEMS applications have more recently driven the design of new specialised package types [66]. Table 1 summarises some of the challenges.

<table>
<thead>
<tr>
<th>Bio-MEMS Applications</th>
<th>Standard Integrated Circuits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Often involve moving solids or fluids</td>
<td>Stationary thin solid structures</td>
</tr>
<tr>
<td>Require integration of microstructures with microelectronics</td>
<td>No such integration is required</td>
</tr>
<tr>
<td>Perform a variety or functions of biological, chemical, optical and electromechanical nature</td>
<td>Transmit electrical signals only</td>
</tr>
<tr>
<td>Many components are required to interface with working media and hostile environments</td>
<td>Integrated circuit die are protected from working media by encapsulation</td>
</tr>
<tr>
<td>Fewer electrical connections and leads</td>
<td>Large number of electrical connections and leads</td>
</tr>
<tr>
<td>Lack of engineering design methodology and standards</td>
<td>Well-established design methodology and standards</td>
</tr>
<tr>
<td>Packaging technology is in its infancy</td>
<td>Mature packaging technology and clearly defined roadmaps</td>
</tr>
<tr>
<td>Assembly is primarily manual</td>
<td>Highly automated assembly techniques available</td>
</tr>
<tr>
<td>Lack of quality and reliability testing standards and test facilities</td>
<td>Mature standards and established quality and reliable testing facilities</td>
</tr>
<tr>
<td>Distinct manufacturing techniques for each application</td>
<td>Manufacturing techniques are proven and well documented</td>
</tr>
<tr>
<td>No industrial standards to follow in design, manufacture, packaging and testing</td>
<td>Well-established methodologies and procedures</td>
</tr>
</tbody>
</table>

Table 1. Summary of MEMS packaging requirements versus standard integrated circuit packaging (Adapted from [67]).

However, there are still many applications where suitable solutions are sparse, especially for MEMS-based biosensors or ‘Bio-MEMS’ [68]. Applications based on semiconductor ICs are such an example, requiring demanding packaging solutions not readily available commercially. For instance, a cell-based IC biosensor raises unique difficulties where the sensor on the semiconductor die must contact cell culture media but simultaneously provide biocompatible electrical and chemical isolation from the bondpads and bondwires at the edge of the die.
Flexible assembly processes can accommodate the bonding of multiple components, including die and discrete components onto a single substrate to form multi-chip-module (MCM, Figure 6a). Similarly, components can be assembled into a single package resulting in a system-on-chip (SoC). Standard packages can be used as a basis for the encapsulation, such ceramic dual-in-line pin (DIP), plastic DIP (PDIP), QFN, QFP, SOIC and SSOP outlines [69]. As an alternative to silicon, lower-scale integration is achievable on a range of flexible (polymer) or rigid (e.g. glass) substrates by depositing layers to form thin film transistors (TFTs).

Figure 6. Example packaging technologies frequently adapted for MEMS applications. a: multi-chip-module, ‘system-on-chip’ (From [70]; Scale bar is 5 mm); b. open-cavity package, shown without die and bondwires (From [69]; Scale bar is 1 mm).

With the industry in its infancy, several prototyping solutions for IC biosensors have been developed by researchers to meet their specific needs [71]–[73], but presently there are no standards. Companies such as Sempac, U.S. [69] and Quik-Pak, U.S. [74] provide custom packaging solutions (Figure 6b) but these are based on semiconductor package outlines and are based on materials which may not be suitable for the biosensor applications (e.g. moulding compound biocompatibility, flexible substrates for use in-vivo).

1.6 Summary
Biosensors are a keystone for neuroscience and provide the artificial inputs and outputs needed to comprehend and interface to biological systems. Novel sensor design has always been at the heart of neuroscience, including the voltage and current clamp circuits used by
Hodgkin and Huxley shortly after the Second World War. Biosensors have continued to evolve since that time and have found application in the diverse fields of neural prosthetics, improved food safety, tools for drug discovery, defence against biological warfare and further innovative tools to assist neuroscientists in their research.

The accepted categorisation of biosensors has been explored and comprises two elements that are usually distinct: the bioreceptor and the transducer. Bioreceptors may be formed of structures of differing scales – from enzymes and DNA through to whole cells. Transducers are broadly categorised into electrochemical-, optical-, and mass-based. Cell-based sensors are of great interest as they generally respond most naturally to the test substances (analytes) they are exposed to. Projecting the likely response of a whole organism to the same analyte is therefore more accurate that extrapolating the effects of lower-level responses at the sub-cell level (e.g. changes in pH or adhesion of proteins).

Multiple electrode arrays (MEAs) have been developed as cell-based sensors for direct interfacing with brain slices or dissociated neurons. Commercial devices are passive components that are custom-fabricated for the purpose. These MEAs are presently expensive, have short lifetimes and have no ability to process the recorded signals. As a result of these shortcomings, analysis of the literature confirms there is great interest in the making the electrode more intelligent by marrying integrated circuit technology with cell-based biosensors. The predominant IC technology is complementary metal oxide semiconductor (CMOS) which is readily accessible to the designer, extremely cheap when manufactured in volume and with which both analogue and digital circuits can be formed.

The approach and difficulties of using CMOS as biosensors have been briefly introduced, including the interface of silicon-based transistors to nerve cells, the ability to adopt the CMOS metal layers at the chip surface as electrodes, and the issues with adapting IC packaging technology for biosensors.

This chapter has therefore served to introduce the concept of the biosensor and has explained the role of CMOS IC technology within this context.
### Key Points

- Biosensors are formed by two distinct elements: a bioreceptor and a transducer.
- Cells are appealing as bioreceptors as they respond most naturally to their environment.
- Multiple electrode arrays (MEAs) are cell-based sensors for interfacing with brain slices or dissociated neurons.
- Commercial MEAs are generally expensive and have short lifetimes.
- MEAs and other biosensors can be formed from CMOS integrated circuits but limitations of aluminium pads and packaging must be overcome.
2 Principles of Cell-Based Biosensor Technology

The design of cell-based biosensors is exceptionally multi-disciplinary and rests on a working knowledge of biology, biochemistry, electrochemistry, material science and electronics. Within these broad fields further expertise is required, such as in electrophysiology, electrode processes, corrosion science and semiconductor fabrication. This chapter will introduce these fields in the context of cell-based biosensors, with a CMOS electrode operating either as a transducer for extracellular action potentials or as an electric cell-substrate impedance sensor (ECIS).

As a pre-requisite to understanding cell-based biosensors, the physiology of cells is outlined in the following section. This covers fundamental principles of cell membrane biochemistry and an overview of electrical excitation.

Secondly, the aspects common to both type of transducer, namely cell adhesion, the metal-solution interface and biocompatibility are outlined: these are all interrelated since it will be shown that a cell must be in intimate contact with an electrode for neuronal recordings, having changed its morphology from conceptually spherical and motile\(^8\) to flattened and adhered. It will be demonstrated later (Chapter 6) how the adhesion of the cell is dependant on the electrode surface morphology and that cell vitality is reliant on a biocompatible environment which requires chemically stable electrodes.

Thirdly, the principles of extracellular neuronal recordings are outlined, explaining how an action potential can be detected non-invasively using a planar metal electrode. This includes a discussion of an electrical model for the cell-substrate interface in the presence of an action potential.

Fourthly, the ECIS transducer is reviewed, again in the context of CMOS technology. By this point the requirements for an intimate contact between cell and substrate will have been explored. To correctly site dissociated motile cells onto the electrodes the technique of

\(^8\) Motility is the ability for a cell to move (migrate) without the application of an external force.
dielectrophoresis (DEP) will be outlined. DEP technology used alone does not form a sensor, but is a supporting technology for cell-based biosensors.

Lastly, a closer look at CMOS processing is necessary, not only to understand the chemistry of the electrode, but also to gain an appreciation of what is truly manufacturable using standard CMOS fabrication processes.

2.1 Cell Physiology

The electrical properties of cells cannot be understood without a foundation in basic cell biology. It is the cell physiology – the physical and chemical functioning – that is key to building cell-based biosensors. Of primary importance here is the boundary that defines the cell – the cell membrane.

2.1.1 Cell Membrane

Membranes of biological cells are formed of a bilayer of phospholipids [75]. These have a central glycerol component with a phosphodiester group at one end and long alkyl tails at the other (Figure 7). They have both polar and non-polar components: the alkyl tails are non-polar and therefore hydrophobic. The phosphodiester group are polar and hydrophilic. These can spontaneously form into bilayers (Figure 8) due the hydrophobic effect [76]. A key property of these bilayers is that they are generally impermeable to water and ions which allows containment of these components inside the cell along with other molecules and structures.
Within and on the cell membrane reside proteins of various types and functions. Proteins, also known as polypeptides are chains of amino acids and can comprise either a single or many chains [75]. The position of polar and non-polar amino acid residues determines the overall shape of a polypeptide (e.g. folding in three dimensions) and determines a protein’s function.

The functions of proteins within cell membranes are extensive, but here, and in the context of this work, the groups forming protein channels, integral proteins and glycoproteins are considered. The integral proteins and glycoproteins are discussed in the next section with regards to cell adhesion, but firstly, the protein channels are considered.
2.1.2 Nernst Equation

Fundamentally, the protein channels within the lipid bilayer form a semi-permeable membrane between the fluid outside of the cell (extracellular) and the cytoplasm inside (intracellular). The ability for a cell to pump ions from one side of its membrane to another creates an electric potential gradient.

The usual textbook approach [79] is to consider a semi-permeable membrane with a high concentration of a given generic ion species on one side, ‘Side A’, and a zero concentration on the other, ‘Side B’ (in this context, these represent the intracellular and extracellular sides of a cell membrane). Under these conditions, ions will diffuse from Side A to Side B. This ion migration therefore also moves net electrical charge from Side A to Side B; this separation of charge creates a potential difference which opposes further net migration of charge. This process is therefore self-limiting and creates a dynamic equilibrium. In this equilibrium state there is an ion concentration gradient across the membrane and also an electrical potential difference. Slightly more formally, the dynamic equilibrium can be considered the point at which the energy gained by movement of the ions down the concentration gradient is equal to the work done against the potential gradient.

Consider any positive ion species, $X^+$, with concentration on Side A, $[X]_A$ and on Side B, $[X]_B$. The electrical potential created by this concentration gradient is $kT \ln \left( \frac{[X]_A}{[X]_B} \right)$, where $k$ is Boltzmann’s Constant and $T$ is absolute temperature. The electrical potential of the separated charge is $e \cdot E_X$, where $e$ is the fundamental charge and $E_X$ is the potential difference. The equilibrium formed by these gives rise to the Nernst Equation (1), where $X$ is any ion species and $z$ is its ionic charge (e.g. $z = 2$ for Ca$^{2+}$) [79]:

$$E_s = \frac{kT}{ze} \ln \frac{[X]_A}{[X]_B} \tag{1}$$

Now consider a membrane with (protein) channels that are ion-selective. This results in an equilibrium that is governed by the respective permeabilities for each ion species. For
example, consider a permeability for potassium, $P_K$, and for sodium, $P_{Na}$. The equilibrium is then defined by the Goldman Constant Field Equation, \( E = \frac{kT}{e} \ln \frac{P_K [K]_A + P_{Na} [Na]_A}{P_K [K]_B + P_{Na} [Na]_B} \) \tag{2}

It can therefore be seen that the potential across a membrane (through which the electric field is constant) will generally be determined by the differences of concentration for each ion species.

### 2.1.3 Resting Potential and Excitable Cells

The potential within a cell is not passively defined by the Goldman Constant Field Equation but is also influenced by the constant activity of ion pumps which move ions from one side of the membrane to another. The energy required for most of these pumps is taken from ATP (adenosine triphosphate). This ‘resting potential’ is governed primarily by the concentrations of Na\(^+\), K\(^+\), Ca\(^{2+}\) and Cl\(^-\) ions [80] and is approximately \(-65\) mV for mammalian cells. However, cells such as neurons (nerve) and myocytes (muscle) can alter their cell potentials by responding to electrical or chemical triggers. This understanding was established during the start of the 20\(^{th}\) century by Julius Bernstein, postulating that the firing of a neuron is a change in the membrane resistance with a flow of ions in and out of the cell [81]. These phenomena were studied during the 1940’s and 1950’s by Hodgkin, Huxley and Katz, leading to the first model that correctly identified the separate and combined functions of sodium and potassium channels during cell excitation – the ‘action potential’. The four-part paper by Hodgkin and Huxley [82] used intracellular probes and electronic feedback circuits to clamp and control voltages within the squid giant axon. We now know that the time- and concentration-related changes of ion current observed by Hodgkin and Huxley are due to the voltage-gated response of protein ‘ion-channels’ that bridge the membrane of excitable cells.

### 2.1.4 Neurons and Action Potentials

The neuron can be distinguished by four elements: the cell body, axon, dendrites and axon terminals (Figure 9) [83]. The body or ‘soma’ contains the nucleus and is the location for most protein synthesis. A single axon extends from the soma and transfers a signal along its
length by a propagating action potential. Terminals at the end of the axon are the outputs connected either to other neurons or to muscle. Connected into the soma are usually many dendrites which act as signal inputs from other neurons. These transfer the electrical impulses through the soma. Between the soma and axon is the ‘axon hillock’ where the cumulative effect of the signals from the dendrites determines whether an action potential will be initiated in the axon.

![Figure 9. The neuron: a. multipolar neurons receive signals from many other neurons at their dendrites and transfer the signal to a single axon; b. motor neuron stimulating muscle; c. sensory neuron showing a branched axon, one branch receiving signals from the sensory cell. The cell body, a dorsal root ganglion adjacent to the spinal cord in mammals, transfers the signal to the second axon branch which is connected to the spinal cord or brain. The arrows show the direction of signal propagation. The motor and sensory neurons of the peripheral nervous system are shown with myelin sheath and Nodes of Ranvier (See discussion on page 25). From [83].]

As previously mentioned, the opening and closing of Na\(^+\) and K\(^+\) ion channels in the neuron membrane are responsible for the action potential. In the last of their series of 1952 papers [84], Hodgkin and Huxley summarised the ion currents as individual conductances,

\[
I_{Na} = g_{Na}(E - E_{Na})
\]

\[
I_{K} = g_{K}(E - E_{K})
\]
where $I_i$ are the individual ion currents, $g_x$ are the conductances, $E$ is the membrane potential and $E_x$ are the respective ion equilibrium potentials. These describe an instantaneous measure of ion currents, as shown in the equivalent circuit of Figure 10.

![Figure 10. An equivalent circuit of a neuron membrane [80] showing the variable conductances $g_{Na}$ and $g_{K}$. The additional $I_L$ component is the leakage current through ion channels that are constantly open. Adapted from [84].](image_url)

However, these ion conductances are dependant on voltage and time. Without an accurate physical model of ion channels, Hodgkin and Huxley were still able to fit the models to describe the variation of $g_x$ versus voltage and time.

The characteristic voltage versus time response of a firing neuron can be explained primarily through the changing permeability of the membrane to sodium and potassium ions. These changes operate in time as well as in distance – i.e. they propagate along the length of an axon. The phases of an action potential are shown in Figure 11: A stimulus from the dendrites (or in the context of biosensors, a stimulus by artificial means) disturbs the membrane potential from its resting potential. Small stimuli have no effect. However, beyond a particular voltage threshold sufficient voltage-gated Na$^+$ channels open to overcome the leakage current. At this point, the Na$^+$ current itself (with ions moving into the cell) is sufficient to cause a further increase in intracellular potential (depolarisation) – i.e. the membrane potential decreases. This is therefore a positive feedback process by which an increasing proportion of Na$^+$ channels open. This process continues until the membrane potential is approximately +40 mV at which time the Na$^+$ channels begin to close. However, voltage-gated K$^+$ channels
now also open but, as observed by Hodgkin and Huxley, have a longer time constant. The $K^+$ current – an outflow of ions – causes the cell to repolarise.

![Figure 11. Phases of an action potential.](image)

The above overview describes the action potential in the time domain for a given location along an axon. However, the action potential also propagates along the axon due to the effect of localised ion currents on the immediately adjacent section of the axon: the depolarisation of a given section causes the membrane voltage of the adjacent section to also decrease. Propagation is assured assuming that the depolarisation of the adjacent section is above the threshold voltage. Propagation along an axon is unidirectional, away from the soma, since lengths of axon that have just completed an action potential take time to recover (the refractory period) before being able to fire again. Additionally, propagation is usually
enhanced in the nervous system by a coating of myelin. This effectively transforms the method of conduction into a transmission line, where the myelin coating increases the ‘line’ resistance and decreases the capacitance. This enables faster transmission of a signal along the axon. However, the signal propagates only as long as the membrane depolarises above the threshold voltage. To ensure this requirement is met, unmyelinated nodes (Nodes of Ranvier) along the axon length act to re-amplify the magnitude of the action potential [80]. A more comprehensive description of the action potential can be found in [79],[80] and [85].

Before developing the above description of an action potential into the principles of extracellular recording (Section 2.5.1), the mechanisms underpinning such an extracellular interface must be introduced: cell-substrate adhesion, the metal surface and its biocompatibility.

2.2 Cell Adhesion

Locations of contact between adjacent cells or between a cell and the extracellular matrix (see below) are cell ‘junctions’, of which there are several types [86]. Occluding junctions form a seal between cells that can prevent even small molecules from transferring through the junction. These are typically found in epithelium (surface-forming) cells. The nature of these junctions and their ability to alter electrical characteristics will be of interest later when considering ECIS biosensors. Anchoring junctions provide cell-to-cell or cell-substrate interaction and are used to mechanically attach cells. These are of most interest here to neuronal and ECIS interfaces as they form the basis for extracellular adhesion. Communicating junctions are the basis for chemical or electrical transference of signals but, whilst being the basis for neuron-to-neuron and neuron-muscle communication, these cellular components (e.g. synaptic junction) are not commonly adopted for artificial communication using neuronal stimulation and recording electrodes.
An essential role in all cell interactions is played by the extracellular matrix (ECM) [86]. The ECM is formed by secretions from the cell itself and is composed of many families of macromolecules, all with differing functions. The matrix forms a supporting framework, binding adjacent cells together and is therefore a basic requirement for multi-celled organisms. In the nervous system, the ECM controls neuron development, survival, migration, axon growth and formation of synapses [88] (Figure 12). Proteoglycans are carbohydrate polymers which attach to other ECM proteins. One function of the proteoglycans is that they hydrate cells due to their negative charge which attracts water molecules. These create a low density region around the cell ranging between tens to hundreds of nanometres in depth, but typically 50 nm [89], and form the glycocalix⁹. The integrins (i.e. integral to the membrane) are used for a diversity of signalling functions: other molecules, including ECM components and other proteins, bind to the integrin receptors (as ‘ligands’) and transfer information about the cell environment into as well as out of the cell. These intrinsic membrane proteins are stated in [89] as protruding between 10–20 nm. Collagen is a family of fibrous glycoproteins that create rod-like structures and provide structural strength. Other fibrous proteins include laminin and fibronectin and both are known to play an important role in cell adhesion and are members of a large family of cell adhesion molecules (CAMs). Specific domains of CAMs

⁹ glycocalyx is an alternative spelling
are usually responsible for the adhesive function, an example being the RGD peptide sequence\(^{10}\): this same fragment is found as domains in both laminin and fibronectin.

With regards to cell adhesion to an artificial surface such as an electrode, an overview is given in [89] wherein the key mechanisms are described: firstly, it is clarified that mammalian cells carry a net negative charge (typically \(-0.024\) C m\(^{-2}\); see Section 2.1.3) and this might suggest that simple electrostatic repulsion would prevent cell adhesion. However, in physiological media this charge is mainly neutralised by counter-ions forming a double layer with a characteristic length given by the Debye-Hückel formula [90].

Since the aim is to promote cell adhesion to an electrode surface, it is necessary to consider the characteristics of the metal surface.

### 2.3 The Metal Surface

Prior to developing a theory involving the modification of CMOS electrodes, it is necessary to understand the surface which is naturally presented by aluminium pads to physiological solutions and cells. The nature of aluminium is determined by its physical and chemical characteristics which are fundamental to its performance as an electrode material. The environments which are of most interest here are aluminium in air, water and physiological media.

Aluminium, along with other metals used in medicine such as titanium, is very reactive – its surface reacts spontaneously with air to form a ‘natural’ oxide film of amorphous Al\(_2\)O\(_3\). This reactivity is determined by the Gibbs free energy of formation, being very negative for aluminium (\(-791.15\) kJ·mol\(^{-1}\)) and titanium (\(-888.8\) kJ·mol\(^{-1}\)) [91],[92]. The instantaneous reaction with air results in growth rate proportional to log-time: the thickness forms very quickly to approximately 10 nm after which it is self-passivating, preventing further reaction and film growth [93].

A comparison of surface charge created when a material is brought into contact with an electrolyte can be assessed using the isoelectric point – the pH at which the net surface charge

---

\(^{10}\) RGD = Arginine – Glycine – Aspartic acid (Arg-Gly-Asp)
is zero. The isoelectric point of aluminium is 8.8–9.5 [91], meaning that at physiological pH of ~7.6 the net charge on the surface is positive. This forms the basis of the electric ‘double layer’ (See Section 2.3.1).

The various surface reactions that may occur in a physiological medium are [91]:

i. Hydroxylation/hydration, creating charges at the surface [94].
ii. Adsorption onto the surface of ions from the physiological medium, such as \( \text{Na}^+ \), \( \text{Mg}^{2+} \), \( \text{Ca}^+ \), \( \text{Cl}^- \), \( \text{SO}_4^{2-} \), \( \text{PO}_4^{3-} \), etc.
iii. Incorporation of ions into the native oxide.
iv. Formation of organic or inorganic films on the oxide surface.
v. Dissolution of the surface

The stability of the metal and oxide film in a medium is defined by the electrochemistry of corrosion and is best illustrated using the Pourbaix diagram of Figure 13. It is important to consider the pH not only of the bulk solution but also the localised conditions. The electrochemical potential, \( E \), defined by the Nernst equation, will also vary due to local conditions such as alloying species, defects and contaminants. At low pH, aluminium dissolves to form \( \text{Al}^{3+} \) ions and at high pH it dissolves to form \( \text{AlO}_2^- \), these conditions both being the basis of corrosion. In pure water corrosion should not occur, but in saline

![Figure 13. Pourbaix diagram for aluminium.](image)
physiological medium, Cl\(^-\) chloride ions will be adsorbed to the surface which creates localised acidic conditions (i.e. dilute hydrochloric acid) under which the passivated surface deteriorates leading to corrosion of the underlying metal [95]. This process then accelerates (auto-catalytic) since the aluminium dissolution process causes a further increase in Cl\(^-\) concentration at the corrosion site. Corrosion is discussed further in Section 5.5.3.

### 2.3.1 Metal-Solution Interface

As will be shown later, the electrical characteristic of extracellular electrodes for neuronal recordings is determined primarily by the chemistry of the solid-solution interface. The basis for modern models stem from the theory devised by Hermann von Helmholtz in the nineteenth century. These theories are presented in [92] and [96], as summarised here.

The electrical double layer is the premise of Helmholtz’s model. The model is built by considering a solid surface with fixed surface charges (Figure 14a). Bringing the surface into contact with a solution will cause counter-ions to be attracted to the charged solid surface. These ions may be either unhydrated ions such as Na\(^+\) or hydrated ions such as Na\(^+\).n(H\(_2\)O). Due to the small but finite diameter of these ions, the metal and counter-ion charges are considered separated by a distance that is equivalent to half the diameter of the ions. This separation of charges therefore represents a capacitance. The capacitance, C, is related to the charge density and the medium by the relationships of Eqn (4) and Eqn (5):

\[
C = \frac{\varepsilon_0 \varepsilon_r A}{d} \quad (4)
\]

\[
V = \frac{\sigma d}{\varepsilon_0 \varepsilon_r} \quad (5)
\]

where \(\varepsilon_0\) is the permittivity of free space, \(\varepsilon_r\) is the relative permittivity, \(A\) is the area of the metal surface, \(d\) is the effective separation of the solid and solute charges, \(V\) is electrical potential and \(\sigma\) is the charge density (per unit surface area).
A refinement of the Helmholtz model is made by the Gouy-Chapman theory. Their model considers that instead of a homogeneous layer of fixed ionic charges, this layer is disturbed by thermal mixing and so produces a ‘diffuse layer’ of net charge which diminishes exponentially with distance from the Helmholtz plane into the bulk of the solution. The voltage, $V$, as a function of distance from the metal surface can be expressed as:

$$V(x) = \frac{\varepsilon_0 K}{\sigma} e^{-\kappa(x-a)}$$  \hspace{1cm} (6)$$

where $x$ is the distance from the metal surface, $\kappa$ is the ‘Debye length’ which defines the rate of decay, and $a$ is the distance of charge separation at the surface. The Debye length is proportional to ion concentrations and therefore the voltage gradient (electric field) is greater at higher concentrations.

Further refinements to the Gouy-Chapman theory can be made by improved derivation methods (giving the Grahame Equation) and by the work of Otto Stern whereby the interface is defined by three layers – the ‘Inner Helmholtz Plane’ (IHP) the ‘Outer Helmholtz Plane’ (OHP) and the ‘diffuse layer’. The IHP and OHP are defined by a linear potential gradient to
distance $a$ and combined are called the Stern Layer. The diffuse layer (also known as the Gouy-Chapman layer [97]) is defined by an exponential drop as previously described by the Grahame Equation (Figure 14b). It should be noted that the model applies for any solid-solution interface. For example, the charge on the membrane surface of mammalian cells has been measured to be negative (approximately $-0.02 \text{ C m}^{-2}$), mainly due to charges on phosphatidylserines. This density represents a single elementary charge, $e$, distributed one per $\sim 10 \text{ nm}^2$ [98].

The double layer and the chemical properties discussed above are key considerations that are developed throughout the remainder of this work (Sections 2.5 – 9).

2.4 Biocompatibility

Any CMOS electrode in contact with biological cells, tissue and/or physiological medium needs to be compatible with its environment, must not alter the physiology of the analyte under investigation or being detected and must not be toxic to any of the biological components in the system. Unfortunately, one difficulty with assessing biocompatibility of IC materials is that no definitive list has been compiled [99] and therefore results must be taken from more loosely related applications such as orthopaedics or smaller evaluations.

Without modification of the CMOS pads, the surface presented by nearly all CMOS technologies is aluminium. The biocompatibility of aluminium and alumina has been thoroughly studied, much work having being done to evaluate in-vivo performance of alumina for use with orthopaedic prosthetics [100],[101]. The performance of aluminium also depends much on the adherent superficial (native) oxide layer and corrosion. However, the in-vivo use of alumina has generally been confined to orthopaedics because to the metal’s poor compatibility with blood due to its thrombogenic action (i.e. its tendency to cause undesirable clotting). Frequently, aluminium is coated with titanium nitride to improve performance [100].

The overall interaction of a prosthetic with its environment is primarily governed by the natural chemistry of the body: simplistically, this is a NaCl aqueous solution of concentration $\sim 0.1 \text{ M}$ with organic acids, proteins, enzymes, macromolecules, electrolytes, dissolved oxygen and nitrogenous compounds. The resulting pH is approximately 7.2, often decreasing
to ~5.5 in the vicinity of tissue damage. Forms of interaction of a prosthetic include inert/bioinert (no reaction), biodegradation (gradual breakdown by biological or biochemical processes), bioresorption (removal by cell activity or by continuous ionic diffusion) and bioactivity (a specific behaviour of a material).

The degradation of the native oxide, alumina, in a physiological environment is limited by its natural corrosion resistance due to the metal being in its highest oxidation state. However, a concern is that either defects in the oxide film may enable aluminium ions to leach into the body or that the alumina itself may degrade. In physiological conditions aluminium easily forms an insoluble Al(OH)_3 precipitate or a solution of AlCl_3. The toxicity of these and other aluminium salts (10–100 mM) has been evaluated and shown to have only a small effect on the viability of mammalian neuronal cells [102]. Additionally, research into Alzheimer’s Disease has not shown a causal relationship with aluminium [103]. Walpole et al. [204] and Karlsson et al. [104] tested nanoporous alumina substrates for aluminium ion leakage and found the dissolution rate of ions into culture measured after 9 days was sufficiently low to be concluded as non-toxic. In the context of IC materials, an in-vitro assessment in [105] showed an enhanced proliferation (vitality) of Caco2 epithelial cells on aluminium versus the glass controls. These results therefore suggest that aluminium with a stable native oxide may form a biocompatible surface.

Whilst we are primarily interested in the CMOS electrodes, the biocompatibility of the surrounding material must not be overlooked. CMOS ICs predominantly use silicon nitride as the surface (passivation). Receveur et al. [99] concludes that silicon nitride is biocompatible, as stated by references therein [106]–[108]. In [105] it was shown that silicon nitride was an excellent substrate for Caco2 cell proliferation.

### 2.5 Neuronal Interfaces

There are many areas of biomedicine that are driving developments in the stimulation and recording of neuronal electrical activity. Applications are primarily drug discovery pharmacology, neural interface systems, cell-based biosensors and systems to assist in the understanding of neural network behaviour. Techniques are available that span the scale of (spatial) resolution: whole brain imaging is possible through methods such as
electroencephalography (EEG), positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) [109]. Populations and networks of neurons can be observed using voltage-sensitive optical dyes that provide response times (temporal resolution) usually into the millisecond range although recent progress has extended the resolution into the sub-millimetre range [110]. However, dyes can be toxic, the dye metabolite\textsuperscript{11} can be toxic, or strong illumination can cause photodynamic damage [111]. Single neuron recordings may be non-invasive but provide only very limited information from a small region of space, typically 10–50 µm [112]. As an extension to the methods of Hodgkin and Huxley, the patch clamp retains the benefit of excellent temporal resolution, effectively unlimited by the fast response times of electronic measurement instruments. The patch clamp is therefore an excellent method for electrical stimulation and recording of single cells but, being an invasive method (with the clamp damaging the cell membrane), the recording duration is usually limited to a few hours.

Figure 15. The patch clamp, a standard technique in electrophysiology for measuring and influencing the intracellular potential and currents of cells and for studying ion channels. A glass micropipette (i) is clamped to the membrane (ii) of a cell (iii) using a vacuum to enclose one or more ion channels (iv). This forms a seal with a patch of membrane inside the micropipette. The micropipette has an internal electrode for measuring and/or controlling potential or current of the patch. The clamp can either remain on the outside surface of the cell, as shown here, or can puncture the membrane to enter the intercellular space (From [78]).

\textsuperscript{11} The products of metabolism
It can be seen from Figure 16 that there is a lack of techniques that provide a spatial resolution enabling recording from one or more neurons but with a temporal resolution than allows action potential recordings (sub-millisecond) for many days. This is important for monitoring a range of biological processes in single neurons or networks such as drug tolerance, neurotoxicity, neurodegradation, network development and activity, including learning, memory and circadian rhythm [113]. This gap in techniques can be filled by non-invasive extracellular electrodes, and the ability to lay out an array of electrodes on a surface for greater spatial coverage makes IC technology a good candidate for this purpose.

2.5.1 Principles of Extracellular Recordings

The principles of extracellular neuronal recordings using planar IC electrodes have evolved from earlier methods using metal wire and glass micropipette electrodes. An electrical model describing the parameters for extracellular metal electrodes and an equivalent circuit was described in a 1968 paper by Robinson [114]. In 1981 the work of Jobling et al. [115]
demonstrated the ability to integrate transistor amplifiers adjacent to an array of microelectrodes on a silicon substrate, capable of recording action potentials from rat brain slice in-vitro. In 1991 it was demonstrated by the Max Planck Institute of Biochemistry that a cell could be directly interfaced to a silicon field effect transistor (FET) [116], where a single mammalian neuron cell formed the transistor gate in direct contact with the silicon channel (switching region) and was stimulated with an intracellular electrode. Most importantly, an electrical model of the junction was developed that remains the basis for all such extracellular planar IC electrodes. Firstly, the concept of a ‘cleft’ between cell and substrate was adapted from the understanding of the synaptic cleft and secondly, the concept of a ‘seal resistance’ was taken from glass pipette patch electrodes. However, for planar extracellular electrodes the physical form giving rise to these parameters is somewhat different from wire electrodes, as illustrated by Figure 17. This is a simplified electrical model showing bulk components as opposed to a more complex model that may represent distributed components: the intracellular potential, $v_{cell}$, is coupled to the electrode via the cell membrane impedance, $c_m$ and $r_m$: the seal resistance element, $r_s$, represents the lateral leakage path from the extracellular space below the cell membrane to ground. (The extracellular medium is assumed to be grounded through a bath electrode.) These electrical characteristics have been investigated elsewhere [117]–[120]. Maximising resistance $r_s$ is critical to forming a good electrical junction between cell and electrode, as demonstrated in [121]. To achieve this without using mechanical manipulation of cells, the height of this ‘cleft’ must be minimised by good cell adhesion. As previously discussed in Section 2.3.1, a double layer impedance is formed at the solid-solution interface for which a simple equivalent circuit is usually sufficient and comprises of a capacitance $c_d$ and resistance $r_d$ [114]. With action potential characteristic frequencies, $f$, in the order of 1 kHz it has been shown that the magnitude of the impedance, $2\pi f c_d^{-1}$, that results from the double layer capacitance typically dominates resistance $r_d$ by a factor of $10^3$ to $10^5$ [122],[123].
2.5.2 Electrical Model of the Electrode

From the above description it is clear that the performance of a planar extracellular recording electrode is strongly dependant on the characteristics of the cell-substrate junction. Figure 18 illustrates a model representing a planar electrode, such as could be formed with CMOS metal pads, with the electrode surface in contact with an electrolyte and partially covered by a cell. The voltage source $V_1$ represents the action potential across the attached membrane. $C_m$ and $R_m$ together represent the cell membrane impedance (c.f. Figure 10). $CPE$ and $R_{ct}$ represent the electrode interface under the attached membrane. $R_{seal}$ is the seal resistance along the cell cleft which is grounded via the solution resistance $R_{sol}$. The area of the electrode exposed to the bulk solution is represented by $R_{ct2}$ and $CPE_{2}$ in series with the solution resistance $R_{sol2}$. The signal detected at the input of the FET is further attenuated by the gate input impedance $R_{in}$ and $C_{in}$, with the resulting signal amplitude represented by the voltage at probe $V_2$. It should be noted that this FET input is simplified and does not include various stray capacitances associated with the FET and track. The model also omits electrostatic discharge (ESD) protection devices that might be required to avoid damage to the ICs whilst being handled prior to use.
Values for the model have been compiled from various sources: most consistent are values for the membrane capacitance, with $C_m \approx 1.0 \, \mu F \cdot cm^{-2}$ \cite{64,118,124}. The electrode area represents circular pads of 30 $\mu m$ diameter. From \cite{125}, seal sheet resistance$^{12}$ is $14 \, M\Omega \cdot \square^{-1}$ and, for rat neurons, the ratio of attached to free membrane, $\beta$, is 0.5 and a cell radius of 3.3 $\mu m$. These values are also consistent with \cite{126}. Values for $R_{ct}$ and $R_{ct2}$ were scaled from preliminary experimental data, the final results being presented in Chapter 9.

Figure 18. Electrical model of electrode and attached membrane, as depicted in Figure 17. The model represents a cell in contact with a planar electrode via the electrolyte in the cleft and where the electrode is only partially covered by the cell. The electrode is shown connected to a single FET gate input. Voltage source $V_1$ represents the action potential across the attached membrane; $C_m$ and $R_m$ is the membrane impedance. $CPE$ and $R_{ct}$ represent the electrode interface under the attached membrane; $R_{seal}$ is the seal resistance along the cell cleft which is grounded via the solution resistance $R_{sol}$. The area of the electrode exposed to the bulk solution is represented by $R_{ct2}$ and $CPE2$ in series with the solution resistance $R_{sol2}$. The signal detected at the input of the FET is further attenuated by the gate input impedance $R_{in}$ and $C_{in}$, with the resulting signal amplitude represented by the voltage at probe $V_2$. The model was created using Orcad Capture CIS v.12.

The capacitors labelled as CPE components are approximations for ‘Constant Phase Elements’: in electronic conduction, passive components can be represented by resistances having a phase angle of zero, inductive elements with a phase of $90^\circ$ and capacitive elements with a phase of $-90^\circ$. However, many real-life systems such as those comprising a solid-solution interface have been found experimentally to contain elements with various phase angles which cannot be represented by such electronic components. A more detailed discussion of constant phase elements is presented in \cite{127} but the differences between a

$^{12}$ measured in ‘ohms per square’
purely capacitive and a CPE are illustrated by contrasting Eqn (7) and Eqn (8): the complex impedance, \( Z_C \), of a capacitor is inversely proportional to the angular frequency, \( \omega \), and capacitance, \( C \). However, the complex impedance of a CPE, \( Z_{CPE} \), includes empirical parameter, \( n \), which provides for any phase angle and factor, \( Q \), which determines the magnitude. Equation (9) is an alternative expression for CPE, the impedance amplitude represented by the factor \( A \) instead of as a function of \( Q \) and \( n \).

\[
Z_C = \frac{1}{j\omega C} \tag{7}
\]

\[
Z_{CPE} = \frac{1}{(j\omega Q)^n} \tag{8}
\]

\[
Z_{CPE} = A(j\omega)^{-n} \tag{9}
\]

A limitation of using electronic circuit models to represent physical systems is that no provision is made for constant phase elements. However, an acceptable fit to measured data can usually be made using a capacitor instead of a CPE, with errors reducing as \( n \) approaches unity. For example, in [123] the microelectrodes were characterised to have \( n \approx 0.9 \).

The components representing the free electrode area (\( Rct2 \) and \( CPE2 \)) are sometimes omitted from models [128],[174], perhaps assuming that the entire electrode surface is covered by an adherent cell membrane. However, as observed in [129], electrodes are often only partially covered by a cell and therefore a fraction of the extracellular signal is lost to ground via \( Rct2 \) and \( CPE2 \). Practically, this means that signal amplitudes are expected to decrease when small cells are placed on large electrodes, as was indeed observed in [129].

The voltage source \( V1 \) shown in Figure 18 represents an action potential with period, \( PER = 40 \text{ ms} \); pulse width, \( PW = 2 \text{ ms} \); rise time, \( TR = 100 \text{ \mu s} \); fall time, \( TF = 200 \text{ \mu s} \); amplitude, \( V2-V1=60 \text{ mV} \). The voltage source has a ground reference since the known action potential magnitude is across the free membrane (i.e. the unadhered cell top) which is grounded to the
bath electrolyte. As explained in [64], the output, $V_2$, is a differentiated signal due to the series capacitances $C_m$ and $C_{PE}$ that dominate the circuit characteristics (This is also why membrane and double layers are often quoted as pure capacitances of $\sim 1 \text{µF} \cdot \text{cm}^{-2}$ and $\sim 0.1 \text{µF} \cdot \text{cm}^{-2}$, respectively, with the high resistance components ignored). It therefore follows that the $dv/dt$ rise and fall rates of an action potential as well as a spike’s amplitude are important to the magnitude of the detected signal. It can also be seen that the circuit configuration, with two series capacitances, $C_m$ and $C_{PE}$, combined with $R_{seal}$ could potentially result in the detected signal being a second differential of the action potential. The detected signal shape is therefore dependent on the cell membrane, seal resistance and electrode surface. It is concluded in [64] that there is no general signal shape for recordings and amplitudes can be optimised by improving the cell-chip contact, by increasing the seal resistance (e.g. by reducing the cleft height), by using recombinant methods to enhance ion channel density or by reducing noise generated by the electrode and transistor circuits.

A further consideration is whether the interface circuit can provide a stable d.c. bias for the CMOS FET without the need for additional circuitry. This is important since additional components can load the signal further, degrading its amplitude and therefore reducing signal-to-noise ratio. The steady state (d.c.) bias of the FET input node is provided simply by the parallel resistances $R_{in} \parallel (R_{ct2} + R_{sol2}) \parallel (R_{ct} + (R_{seal} + R_{sol}) \parallel R_{m}) \approx R_{ct2}$. Since $R_{ct2} \ll R_{in}$, the FET gate input node is sufficiently biased to maintain a stable d.c. state. The above values are for a half-covered electrode with $\beta = 0.5$. If an electrode was entirely covered by a cell ($\beta = 1$) then $R_{ct2} = \infty$ and the magnitude of $R_{ct}$ halves. With $R_{ct} = 1.6 \times 10^{11} / 2 = 8 \times 10^{10} \Omega$, it holds that $R_{ct} \ll R_{in}$ and therefore the FET input should remain adequately biased at $\sim 0$ V when a cell completely covers an electrode. It can therefore be concluded that it should be possible to configure a CMOS FET gate input so that it has sufficient d.c. bias to avoid undesirable supplementary circuitry under all conditions. The complexity of ESD protection has not been discussed here, but is a necessity in any commercial application in order to prevent damage to the FET gate oxides [130].

---

13 The bulk electrolyte in electrophysiology forms a ‘bath’ above an electrode. The bath is usually grounded by either a wire or plate electrode, or by a planar IC electrode.
The above model is also useful for fitting electrochemical impedance spectroscopy (EIS) data during the development of electrodes (Sections 8 and 9) where it will be discussed how the electrode impedance is a critical design factor.

### 2.6 Electric Cell-Substrate Impedance Sensors (ECIS)

The study of processes such as tumour growth, wound healing, cell migration and the understanding of how cells interact with a substrate can be assisted by measuring cell confluency (i.e. proportion of a substrate covered) and the degree to which cells have formed tight cell-cell junctions and cell-substrate junctions [131]. Further, the ability to measure cell growth and adhesion enables the effects of extracellular matrix protein coatings to be assessed. In 1984, Giaever and Keese [132] demonstrated a non-invasive method that quantifies coverage and adhesion: planar electrodes to which an a.c. electric field was applied could detect changes in impedance when fibroblasts were cultured on them. Such electric cell-substrate impedance sensors have since been developed [120],[133], leading to commercial products, especially as drug development tools. Products include single interdigitated\(^\text{14}\) electrode structures (IDES) and electrodes in microtitre plates with up to 96 wells [32]–[34],[134]. For large numbers of wells, the basic electrodes can be produced cost-effectively by screen-printing [135]: these are then passive devices that require a large number of connections to the plate and have no on-plate circuitry to provide signal processing or recording. Wireless technology has been explored for multi-well plates, but the custom fabrication of electrodes that was used does not lend itself to producing low-cost commercially-viable plates [136].

The principle of ECIS is simply the measurement of impedance across a layer of adherent cells. Measurements can be taken in real-time and can monitor the growth of cells as they spread across a planar electrode substrate (Figure 19a). A bare electrode is covered with physiological medium of high conductivity (electrolyte) which forms a low impedance circuit between the ECIS electrode and a counter electrode. The counter electrode can be either a second planar electrode on the substrate or an immersed bath electrode. When the electrode is covered by cells, the high impedance of the cell membrane causes the system impedance to increase. The response to cell adhesion is not detectable at d.c., but is more readily detected

\(^{14}\) Interdigitated is where two electrodes have inter-leaved long fingers producing a high surface area electrode with a large working area between the two separate electrodes.
between frequencies of $10^2$ to $10^5$ Hz (Figure 19b). In [131] it was confirmed that an optimum frequency for ECIS is 40 kHz and that the most significant effects are measured by monitoring the imaginary (capacitive) component of the complex impedance. Such analysis of impedance is achievable using bench analysers or can be readily implemented in CMOS circuitry. The technique is also sufficiently sensitive to detect the motility of cells to a resolution of 1 nm, even when the layer is confluent [137].

Figure 19. Principles of ECIS: a. the spreading of adherent cells on an electrode increases the resistance under the cell (forming a cleft) and also increase the resistance between adjacent cells that form tight cell-cell junctions (From [137]); b. EIS data shows a difference between substrates with and without cells for the real impedance (resistance), i, and imaginary impedance (capacitance), ii (From [138]).
It can be seen that a key advantage of ECIS is the ability to measure cell kinetics non-invasively in real-time. Presently this can be performed with ECIS only with a small number of wells per plate, each requiring multiple external electrical connections – one per electrode. As a result, it becomes difficult to perform real-time measurements whilst the plate is in an incubator and so may limit an assay to periodic readouts when the plate is removed from the incubator. Alternatively, a large number of connections could be made into an incubator from external instrumentation, but this introduces complexity and an increased risk of biological contamination. It is therefore conceivable that a stand-alone multi-well plate with on-board recording of cell impedance is attractive both for drug development assays and as a research platform. Such an arrangement can be readily implemented using CMOS ICs, powered either by just two wires per plate or wirelessly. The ability to form ECIS electrodes using CMOS IC technology is therefore part of the scope of the research, with the development presented in Chapter 9 and ECIS experiment results in Chapter 11.

### 2.7 Electrophoresis

The movement of a charged surface relative to a stationary liquid by an applied electric field is known as electrophoresis [139]. A comprehensive review of the use of electrophoresis in the manipulation of cells is provided by [140],[141]. It should be noted that any particle, even with no net charge, can be manipulated by using spatially non-uniform a.c. fields and is the phenomenon known as dielectrophoresis. A summary of electrophoretic processes is provided in Table 2.

<table>
<thead>
<tr>
<th>Field type</th>
<th>Particle type</th>
</tr>
</thead>
<tbody>
<tr>
<td>d.c.</td>
<td>uncharged</td>
</tr>
<tr>
<td></td>
<td>charged</td>
</tr>
<tr>
<td>homogeneous a.c.</td>
<td>no movement (but may induce dipole)</td>
</tr>
<tr>
<td>spatially heterogeneous a.c.</td>
<td>dielectrophoresis (and may induce dipole)</td>
</tr>
</tbody>
</table>

Table 2. Electrophoresis field types and their effect on particles

The phenomena can not only move particles and cells, but can also sort and characterise them due to differing physical and electrical characteristics. The technique therefore has many medical applications. One limitation of present technology is the lack of integration with other
components. This task is a focus of the electrophoresis industry [142] and is perhaps where biocompatible CMOS can demonstrate the ability to provide higher levels of integration.

Negative dielectrophoresis (n-DEP) is where an a.c. field in an electrolyte is designed to manipulate particles into specific locations that have minimal or zero field strength. These are therefore electrophoretic traps that will hold particles. Cells, for example, can therefore be accumulated at these points where there is minimum dielectrophoretic effect. This method has been used in [143] to centre cells above neuronal recording electrodes. It must be noted however that such positioning of cells does not in itself achieve tight junctions: the DEP field does not adhere cells to the substrate and they may therefore remain motile [144].

An example of a cell-based biosensor (in the form of a pH ion-sensitive FET (ISFET)) has been demonstrated by [145] where platinum n-DEP electrodes were designed to position yeast cells and bacteria onto the gate of the ISFET. In [146] four n-DEP electrodes were used to position rat neurons into the centre. Analysis was performed to ensure that the applied field did not cause cell membrane breakdown. It was noted that calculations may depart from the theoretical field strength and cause excessive field strengths but experiments showed that in fact there was no degradation in the viability of the cells.

Figure 20. Positioning of cells using n-DEP: a. An ISFET pH biosensor with cells positioned onto the gate (marked) of the FET (From [145]); b. Neurons manipulated to the centre of four n-DEP electrodes (From [146]).
From the outset of this work, it was appreciated that cells need to be positioned on the electrodes for successful sensor operation. However, since it has been shown above that the principles of DEP are already well understood, this was not to be a focus of this research and DEP was not used.

To design DEP electrodes as well as all sensor electrodes in CMOS requires an understanding of fabrication processes and this is addressed in the following section.

2.8 An Overview of CMOS

CMOS is the prevalent technology used to meet worldwide demand for IC products. It is therefore no surprise that research has attempted to adapt CMOS for other applications such as biosensors. However, CMOS processes have always been purposely developed to be closed to the surrounding environment to avoid contamination problems that lead to low manufacturing yield and poor reliability. Therefore opening the chip surface to form sensors is somewhat inconsistent with the goals of most semiconductor manufacturers. The general structure of a CMOS IC is shown in Figure 21. Two metal layers are shown in this generic example. Transistors are formed within the silicon substrate. The transistor gates are then formed above the FET channel regions. The first layer of metal is then deposited, forming contacts with the transistor source and drain regions. An interlayer dielectric (ILD) is deposited onto the metal. One or more additional metal layers can be deposited, each one insulated from the layers below using additional ILD layers. Windows in the passivation allow connection between adjacent metal layers. A film of insulator, often comprising two separate layers, is deposited over the chip – this is the passivation which protects the circuits from physical damage and from contaminants. The only openings in the passivation are onto bondpads formed from the top layer of metal. The bondpads provide electrical connections to/from the chip. The section shown in Figure 21 has only two metal layers, but modern CMOS processes often have many more metal layers. The processing of these requires flattening of the surface between each metal deposition: these are ‘planarised’ processes and avoid problems of metal and insulator coverage (‘step coverage’). The result of planarisation is that the chip surface is flat, with steps only at the openings of the bondpads (Figure 22). The height variation of passivation in unplanarised processes may be several microns and therefore might be a consideration in positioning of cells on surfaces of CMOS biosensors [147].
Figure 21. General structure of a CMOS integrated circuit with two metal layers (From [148]).

Figure 22. Cross section of a planarised CMOS circuit. The surface of each metal and interlayer dielectric is flat, facilitating easier deposition of additional metal. No bondpads are shown on this section. The surface of the chip is here protected with three passivation layers. (From [149]).
Aluminium forms the conventional basis for high volume IC metallisation and is likely to continue to do so for the foreseeable future.\textsuperscript{15} A typical metallisation stack is shown in Figure 23. The inclusion of a small proportion of copper (typically 0.5\% and up to 4\%) reduces the reliability problem of electromigration \textsuperscript{[150],[151]}. Due to the presence of shallow silicon junctions at contacts (where metal contacts silicon) it is also necessary to prevent ‘contact spiking’ – the eutectic alloying of the aluminium and silicon. This is frequently achieved through the incorporation of a ‘barrier layer’ at the base of the metal stack and by alloying the aluminium with silicon – typically 1 to 2 wt\%.\textsuperscript{16} Typical materials for barrier layers are titanium, titanium nitride and titanium-tungsten \textsuperscript{[151]}. Additionally, it is frequently necessary to include an anti-reflective coating (ARC) on top of the stack to prevent undesirable photolithography problems. This is typically titanium nitride \textsuperscript{[151]}.

![Figure 23. Typical CMOS metallisation (simplified, and for brevity showing only single-layer metal). The thickness of the marked ‘stack’ is typically 1 µm and here comprises a titanium barrier layer, an alloy of Al-Si-Cu and a titanium nitride anti-reflective coating.](image)

Mature CMOS processes that are likely to be used for small quantity production, such as for MEAs, are typically >0.1 µm processes which continue to use aluminium for metallisation.

\textsuperscript{15} for the final metal layer in a process
\textsuperscript{16} ‘wt’ = weight.
For deep submicron (<0.1 µm) processes, the semiconductor industry has transitioned to copper, but this may not be totally complete for niche applications until the 45 nm node is reached [152]-[154]. Even on these more advanced processes, aluminium is often used to coat the final (upper-most) metal layer to ensure high quality bonding.

2.9 CMOS Electrodes for Cell-Based Biosensors

This, the final section before introducing the research thesis in Chapter 3, reviews the past work at the University of Bath in creating CMOS electrodes for neuronal interfaces. This is followed by a concise review of existing CMOS technology, primarily centred on the core application of neuronal recordings. A key aspect of successful recordings is reviewed: the necessity of minimising the cleft between cell and electrode. These factors then provide the context for the author’s own thesis.

2.9.1 Previous Work at the University of Bath

Previous work completed by King’s College London (KCL) and the University of Bath under EPSRC grant GR/S08237/01 investigated recording from NG108-15 and rat dorsal root ganglion (DRG) cells using passive CMOS MEAs and commercial MEAs (Multi Channel Systems GmbH, Reutlingen, Germany). The results are shown in Table 3 which illustrates the 0% success rate for recording from the CMOS electrodes. It had therefore been surmised that aluminium might not be biocompatible and the results had initially seemed to corroborate the literature [102],[155],[156].

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Device type</th>
<th>Experiments</th>
<th>Devices used</th>
<th>Successful recordings</th>
<th>Success rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG108-15</td>
<td>MEA</td>
<td>28</td>
<td>89</td>
<td>5</td>
<td>5.6</td>
</tr>
<tr>
<td>NG108-15</td>
<td>CMOS</td>
<td>5</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DRG</td>
<td>MEA</td>
<td>17</td>
<td>81</td>
<td>15</td>
<td>18.5</td>
</tr>
<tr>
<td>DRG</td>
<td>CMOS</td>
<td>4</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Results of previous work by Taylor et al. [113] studying passive CMOS electrodes.

An objective of this research was therefore to understand better the poor success rate of CMOS electrodes and to develop solutions suitable for commercialisation. A metric of success was therefore the ability to culture cells on electrochemically and electronically stable CMOS electrodes, maintain cell vitality through good biocompatibility and then proceed to
Chapter 2 – Principles of Cell-Based Biosensor Technology

stimulate and record action potentials. Once again it is emphasised that the premise of the work at The University of Bath and King’s College London was to create low-cost CMOS electrodes that would be suitable for commercialisation, i.e. processing of standard CMOS devices without additional photolithography or other costly post-processing steps.

2.9.2 State of the Art CMOS Biosensors

Research into CMOS interfaces for neuronal recordings has centred mainly on two types of electrode: the electrolyte-oxide-semiconductor (EOS) FET and the metal electrode. It is maintained that it is the sensor interface itself that is the primary obstacle to successful products and is a necessary focus of research – other aspects of a CMOS neuronal interface product, such as signal amplification, data processing and communication, can leverage capabilities that are already well-established in the semiconductor industry. In this respect, impressive CMOS MEAs have already been demonstrated [130],[157]-[163] and so the design of CMOS circuitry is not a focus of this thesis.

Firstly, an EOS FET interface has been pursued by The Max Planck Institute of Biochemistry, Munich, (Fromherz et al.) as it offers the potential of providing a first-order (direct) response to the action potential (i.e. FET current proportional to membrane potential) [164],[165]. A drawback is that complex (lithographic) post-processing of the IC is required to form EOS FETs from standard CMOS processes. This is because the CMOS gate oxide layer is below the passivation and all metal layers. CMOS gate oxides are therefore not readily interfaced directly to culture medium and cells. A further concern – little emphasised in the literature – is the likelihood that such an arrangement will be adversely affected by ionic contamination from contact with the culture medium [150],[166]. Drifting of EOS FET voltage thresholds could conceivably be compensated for within an amplifier design, but ionic contamination, being highly mobile, is just as likely to cause rapid functional failures in CMOS logic gates surrounding the electrode array. This may ultimately limit the ability to use this form of EOS FET to produce a commercial product with a useful lifespan.

An adaptation of the EOS FET has been presented in [167] and [168] which improves the passivation of the transistor by connecting the standard polysilicon gate of the sensing FET up to the surface of the IC (Figure 24). The top layer of metal defines the sensor area but this is covered by standard IC passivation to avoid the need for post-processing. This process is
reported to work well when the electrode is configured as an ISFET [167]. However, for cell-based sensors, sensitivity improvements have been necessary by switching the standard, thick (typically 1-2 µm) CMOS passivation to a hafnium high-κ dielectric passivation [124],[169]. The hafnium passivation is still not as sensitive as a metal electrode and it has necessitated the use of large snail neurons in order to demonstrate successfully the capability of this technology in neuronal recordings [124]. Additionally, the hafnium process may re-introduce the need for photolithography to open windows for bondpads. Further, preliminary tests showed the thin (50 nm) hafnium passivates the aluminium bondpads from corrosion for short cell-based assays of 5 days, but it remains unclear how this thin film will perform during longer periods of use.\(^\text{17}\) Conceivably, a thicker uniform hafnium film could be deposited over the whole device after wire bonding and assembly, but unfortunately hafnium deposition is a 250ºC process under vacuum that, whilst compatible with devices at the wafer level, is incompatible with packaged devices.\(^\text{18}\)

![Diagram of FET with annotations](image)

**Figure 24.** A floating gate EOS (Electrolyte-Oxide-Semiconductor) FET. The FET gate, G, is accessed from the top of the IC through the metal layers. The upper metal layer defines the sensitive (electrode) area which is covered by the silicon nitride passivation. Charge above the sensitive area induces a charge on the FET gate which in turn modulates the current in the n-type silicon channel between source, S, and drain, D. (From [168]).

So it seems implementing an EOS FET in CMOS either leads to an ionic contamination hazard or requires post-processing photolithographic steps in a cleanroom to define bondpad windows in the hafnium passivation (which once again places such a process outside the

\(^{17}\) For example, *in-vitro* cell cultures may be up to, say, 56 days [168] and electrodes may be expected to be reusable. Electrode arrays in the ECIS experiment of Section 11 were used for 28 days and the neuronal recording experiments of Section 10 for 70 days.

\(^{18}\) Moulding compounds and elastomer may out-gas or fracture under high vacuum and may decompose at 250ºC.
scope of this work). Indeed the need for post-processing for these FETs has been confirmed in a recent review [170],

‘With an appropriate post-process these [floating-gate FET] devices can be operated in a liquid environment.’

An alternative to the EOS FET has been to pursue the use of metal (usually platinum) electrodes based on standard CMOS bondpads (Figure 25) [157],[160],[171],[172]. However, because of the cleft between cell and substrate and the double layer at the solid-solution interface (Section 2.3.1), this approach in fact also leads to a capacitive coupling. However, where the interface is particularly tight (i.e. with a small cleft between cell and substrate), models illustrate that it may be possible to provide a first-order (ohmic) response since the cleft can be modelled as a resistive component that sinks action potential ion current laterally under the cell into the bulk of the electrolyte [173],[174].

Figure 25. Adaptation of CMOS using photolithographic processing to re-define electrodes using platinum (From [155]). This process requires a microfabrication facility to add additional layers on top of the CMOS IC (and therefore does not meet a low-cost criterion).

Interestingly, despite the difficulties with both the direct and capacitive coupling methods, there are some similarities in their equivalent circuits: both electrical models include a capacitive cleft and both recording electrodes typically connect to a high impedance FET gate. However, as established in [124], the sensitivity of the metal electrode still out-performs
The floating gate EOS FET, with the metal electrode clearly more suitable for recordings from small mammalian neurons.

### 2.9.3 Maximising the Neuronal Recording Signal

As explained in Section 2.5.1, the height of the cleft between the cell and electrode is a key parameter that influences the magnitude of extracellular neuronal signals and has therefore been a key consideration in the development of state of the art sensors. It has been discussed how the cleft resistance is also a key parameter in ECIS biosensors (Section 2.6).

The use of adhesion proteins has been a main line of investigation as a tool to minimise the cleft by forming tight electrode junctions with the electrode/substrate. It was concluded in [175] that the proteins promote cell adhesion and that the cleft can be minimised using a coating of RGD peptide sequence. In [63] it was noted that RGD immobilises cells, with the exception of neurons. Polylysine was also discussed as an adhesion molecule but cleft size was expected to be larger compared to a layer of RGD peptide. In [124] and [174] it is stated that the YIGSR peptide sequence – a laminin fragment – also promotes cell adhesion whilst minimising cleft size. Other methods to promote good adhesion include the use of polyethylenimine (PEI) and laminin [176]. Whilst generally successful at producing adhesion, the cleft is wider and so they produce a less efficient interface (Figure 26).

A MEMS approach to adhesion has also been investigated whereby a wafer was micromachined to provide pneumatic anchoring of rat cardiomyocytes [62]. This technique has been successfully incorporated into a family of single-use MEAs manufactured by Cytocentrics AG, Germany [177]. Investigations by [178],[179] showed that modification of a silicon surface by patterning (in the range of tens of nanometers to micrometers) can also assist with attachment.

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19 RGD = Arginine – Glycine – Aspartic acid (Arg-Gly-Asp) - a laminin and fibronectin fragment
21 Coupled voltage reduces with the square of the cleft distance.
Figure 26. TEM images showing examples of cell-substrate clefts – From [180]. Cells have been fixed and sectioned using a focussed ion beam: a. A platinum substrate (the surface marked with black arrows) was coated with laminin-111 prior to adhesion of chicken embryo neurons. The cleft is between the adhered cell membrane (marked by white arrows) and the platinum surface and was measured to be 27-108 nm; b. L1 Ig6 (the sixth immunoglobulin domain of cell adhesion molecule L1 and known to promote neurite extension) has a lower molecular weight (8 kDa) and is a smaller molecule than laminin-111 (~800 kDa). Smaller molecules generally result in smaller clefts, as illustrated here by the cleft of 26-79 nm.

Silicon substrates have been successfully modified by [181] and [144] to produce porous silicon. Scanning electron microscope (SEM) images suggested a tight junction but these need to be confirmed by electrical characterisations. Unfortunately, the opening of windows to silicon introduces the same drawback as the EOS FET in that ionic contamination of the CMOS logic circuits is likely.

A second key parameter affecting the effective signal of a neuronal recording is the impedance of the electrode. The majority of publications reporting successful recordings from CMOS ICs (c.f. references in Section 2.9.2 above) use a coating on the electrode of platinum black to increase its effective surface area and hence decrease its impedance.\(^{22}\) This is a well-established method used for electrophysiology and other electrochemistry applications [182]. In [129] CMOS IC electrodes were successfully coated with biocompatible platinum and platinum black. The platinum pads were created by a lithographic patterning step, but the subsequent platinum black was an electrodeposition performed by biasing the stimulation circuitry. The platinum black was shown to reduce successfully the electrode impedance.

\(^{22}\) With reference to Figure 18, the electrode impedances CPE // Rct and CPE2 // Rct2 scale with the effective electrode area. Therefore, increasing the effective electrode area by a factor, say, of 10 using a coating of platinum black will decrease CPE, Rct, CPE2 and Rct2 also by a factor of 10.
Interestingly, the benefit arising here from decreased electrode impedance is not intuitive: the improvement comes not from an increase in signal amplitude but instead from reducing the noise produced by the electrode itself — i.e. the benefit is an improved signal-to-noise ratio at the FET gate input. This occurs since the r.m.s. thermal noise, $V$, produced in an electrode is proportional to the root of its resistance, $R$, where, $k$ is Boltzmann’s constant, $T$ is temperature and $B$ is the bandwidth [184],[185], Eqn (10).

$$V = \sqrt{4kTBR}$$ (10)

As outlined in Chapter 1, the potential applications of a silicon-electrode junction are diverse and so the interface to many types of cell is relevant to this work. This needs to be taken into consideration when reviewing recent developments in the literature. For example, many of the published results demonstrating successful recordings from electrically active cells relate not to mammalian neurons but to Human Embryonic Kidney (HEK) cells and cardiomyocytes [117],[118],[129],[160],[174],[176],[186]-[195]. Such cells may often produce signals of larger amplitude than achievable with mammalian neurons. Therefore, producing reliable extracellular mammalian neurons recordings using CMOS electrodes is probably one of the most demanding cell-based biosensor applications.

### 2.9.4 Obstacles to Commercialisation

Commercial cell-based biosensors based on proprietary substrate technologies are not readily scaled to applications requiring large numbers of electrodes, such as drug discovery and multiple electrode arrays for neuronal recordings. However, more scalable technologies such as CMOS have yet to demonstrate reliable operation as cell-based biosensors. Some of the difficulties were discussed in Section 2.9.1. It is the objective of this study to understand better the problems of using CMOS in these environments and to develop solutions that may catalyse commercialisation. Metrics of success are therefore the ability to culture neuronal cells on electrochemically and electronically stable CMOS electrodes, maintain cell health

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23 As explained above in Section [2.5.2] and by [129], the signal magnitude relates to the proportion of an electrode that is covered by a cell, i.e. forming a potential divider. Decreasing the impedance per unit area using platinum black decreases the impedance under the cell but also decreases the impedance to the grounded bulk electrolyte and therefore the platinum black has little direct effect on signal amplitude.

24 r.m.s. is the ‘root mean square’ of the instantaneous noise.

25 Additional sources of noise exist in electrode-electrolyte interfaces [183] but thermal noise dominates in neuronal recording FET-based electrodes.
(vitality) through good biocompatibility and then proceed to demonstrating the recording of action potentials.

Through continuous development of the CMOS interface, particular constraints and techniques are emerging that may lead to successful electrode products and are summarised in Table 4. The constraints are effectively requirements of a commercial interface. The emerging characteristics are defined as the techniques being pursued by the main research groups (as discussed in Section 2.9.2).

<table>
<thead>
<tr>
<th>Constraints (for commercially viable solutions)</th>
<th>Emerging Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>use of standard CMOS technologies (mature processes: low cost; high availability)</td>
<td>bondpad modification using plating</td>
</tr>
<tr>
<td>no complex post-processing of ICs (e.g. no additional lithography)</td>
<td>high spatial resolution (for MEAs) using addressable sensor arrays</td>
</tr>
<tr>
<td>useful lifetimes for long in-vitro assays or long-term (chronic) implantation</td>
<td>minimised cleft by using specific adhesion layer peptides</td>
</tr>
<tr>
<td></td>
<td>improved packaging based on MEMS technology</td>
</tr>
<tr>
<td></td>
<td>robust signal amplification using very low-noise amplifier designs</td>
</tr>
</tbody>
</table>

Table 4. Themes for commercially-viable CMOS neuronal interfaces.

### 2.9.5 Commercial MEAs

As CMOS technology has not yet penetrated the commercial MEA market, it is worthwhile briefly reviewing current suppliers of MEAs for in-vitro neuronal recordings. The principal manufacturer is presently Multi Channel Systems GmbH, Germany (MCS) supplying a range of MEAs with planar microelectrodes, typically an array of 60 recording electrodes, plus an optional large planar stimulation electrode [196]. MCS have developed a comprehensive set of amplification, data acquisition and data analysis tools to support their MEAs product line. As discussed above in Section 2.9.3, MCS have identified that low impedance electrodes are necessary in order to achieve acceptable signal-to-noise ratios and they have therefore developed a high surface area titanium nitride (TiN) electrode that performs a similar function to platinum black and typically achieves an impedance of only 40 kΩ at 1 kHz for a circular electrode of 30 µm diameter (Figure 27). Whilst the electrodes are high performance, their durability is low: MCS state that the electrodes are re-usable but cleaning is difficult as the electrodes cannot be touched with cotton buds, etc, due to the fragility of the TiN. The
electrodes also seem to degrade at ambient conditions and therefore might have a useful maximum lifetime of one year. It is perhaps for this reason that other suppliers use conventional platinum and platinum black electrodes. Ayanda Biosystems SA, Switzerland, manufacture MEAs with a footprint compatible with the MCS data acquisition systems [197]. Ayanda produce square (40 x 40 µm) platinum electrodes with typical impedances of 400–600 kΩ (at 1 kHz). It is notable that the insulation used by Ayanda is SU-8 and this is known to have a lifetime of only approximately 1 week in physiological conditions (See Chapter 7). Alpha Med Scientific, Inc., Japan, manufacture the MED64 system which comprises 64 electrode MEAs and supporting data acquisition and analysis instrumentation [198]. Each square electrode is 50 x 50 µm and coated with platinum black to achieve a low impedance of only 7–10 kΩ (at 1 kHz). It should be noted that this low impedance is achieved at the expense of the platinum black creating tall (7.39 µm) dendritic growths above the substrate surface (Figure 28). It is questionable whether the body (soma) of dissociated neurons will readily cover such a tall feature and instead the cells may prefer to adhere only to the substrate (c.f. discussion in Sections 9.5 and 10).

All three manufacturers use glass substrates which have the benefit of allowing imaging of the cells using phase contrast microscopy. MEAs can be supplied optionally with indium tin oxide (ITO) tracks that are transparent at visible wavelengths and therefore further improve the clarity of optical microscopy. This is not possible with CMOS as the silicon substrate is opaque.

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26 The short lifetime of the TiN electrodes is also supported by anecdotal evidence combined with impedance tests performed by the author on MEAs that were approx 5 years old. The electrode impedance had degraded many orders of magnitude during this period.
Figure 27. A Multi Channel Systems MEA. The magnified images show the array of 64 electrode and further SEM images showing a single electrode and its surface. The lower image shows the high surface area dendritic TiN (From [196]).

Figure 28. MED64 MEA: a. a single 50 x 50 µm electrode showing copious platinum black deposition used to achieve a low impedance; b. profile of a MED64 electrode showing tall (7.39 µm) dendritic growth, mainly at the periphery of the electrode (From [198]).

2.10 Summary

It has been shown how the function of cell-based biosensors is entwined with the physiology of cells. Bio-lipid cell membranes result in an intercellular electrical potential that is governed by the Nernst Equation. The rapid changes in the intracellular potential that can be triggered in excitable cells such as neurons are the basis for nerve signalling: this is the ‘action
potential’. The characteristics of the action potential were first described by Hodgkin and Huxley in 1952 and it has since been discovered how a family of ion channels in the membrane form the action potential.

For neurons to communicate either cell to cell or cell to sensor they must adhere and form tight junctions. Adhesion is primarily controlled by a range of cell membrane proteins (integrins) using a family of cell adhesion molecules (CAMs) and the extracellular matrix (ECM). The electrode of a biosensor must therefore interact with the cell membrane and ECM to form an electrical junction.

Due to the physical height of the ECM and CAMs, a cell on an electrode always forms a cleft filled with extracellular medium. The electrode surface therefore always forms a solid-solution interface, and has an electrical ‘double layer’ as described by the Gouy-Chapman theory: the double layer is dominated by a capacitive element at the frequencies of most interest in biosensor applications.

Any electrode in a biosensor must be compatible with its biological element. An unmodified CMOS device presents aluminium pads that can be used as electrodes. However, the aluminium metal is always covered by a thin native oxide film (alumina). The biocompatibility of alumina has been shown to be good, with a long history of use in orthopaedics. However, the thin native oxide will be shown later to form an inadequate barrier to corrosion in the harsh physiological environment. CMOS electrodes therefore require additional processing after fabrication to form biocompatible and electrochemically stable electrodes. Developing a low-cost and reliable post-fabrication process is stated [199] as being the primary obstacle to commercialisation of CMOS biosensors.

Applications suited to CMOS electrodes include, but are by no means limited to, neuronal recordings, electric cell-substrate impedance sensing (ECIS) and electrophoresis (manipulation of cells using a.c. fields). The development of neuronal interfaces is based on interfacing the cell to a metal electrode, an electrolyte-oxide-silicon (EOS) FET or floating-gate FET. An appraisal of state of the art methods suggests the EOS and floating gate FETs may be most suited to ion-sensitive FETs (ISFET) or applications where integration with CMOS circuits is not required (principally due to the reliability hazard of ionic contamination). The focus for commercially-viable biosensors based on standard CMOS is
therefore the post-processing of the aluminium electrodes. The same requirements are necessary for ECIS and electrophoresis.

An electrical model for the neuron-electrode junction has been explored, and describes how the height of the cleft between cell and electrode is critical in achieving good electrical coupling. A model has been used to explore the characteristics of the interface. Circuit analysis shows that the interface also provides sufficient d.c. coupling to a CMOS FET gate to allow simplified amplifier design.

A more in-depth review of CMOS technology illustrates how passivation is key in preventing ionic contamination of the transistors in the silicon substrate. Also, the aluminium electrode is in fact not pure aluminium – a typical specification being an alloy with about 1% silicon and 0.5% copper. The metal is also comprised of a sandwich where the aluminium alloy is contained within a lower layer (usually titanium or titanium nitride) and an upper layer of titanium nitride. These are necessary for IC reliability and will be re-visited later as critical factors in the investigations and subsequent electrode development.

Previous work by the University of Bath and King’s College London was unable to obtain neuronal recordings using standard CMOS aluminium electrodes. This was the starting point for the work described in this thesis. In addition to considering biocompatibility and corrosion, other factors for success include electrode impedance and the need to produce a tight cell-substrate junction. It has been shown that minimising impedance is necessary to reduce the thermal noise produced by the electrode itself. This improves the signal-to-noise ratio so that the small extracellular neuronal signals can be detected. Increasing the signal amplitude by minimising the cleft is possible by careful selection of adhesion proteins or short-chain peptides used to coat the IC surface before use. Additionally, electrode morphology has been explored, with a preference for cells to adhere to porous silicon instead of the IC passivation. This suggests that morphology is worthy of further investigation as a method to promote cell-substrate adhesion.
<table>
<thead>
<tr>
<th>Key Points</th>
</tr>
</thead>
</table>
| - A neuron is an electrically-excitable cell that transmits a signal via a change in its intracellular potential. This is an ‘action potential’.
| - Neurons require tight junctions to communicate cell-to-cell (and to transducers).
| - Tight junctions depend on cell adhesion molecules (and surface chemistry).
| - Solid-solution interfaces always have an electrical ‘double-layer’.
| - Electrical equivalent circuit modelling helps to understand the principles of extracellular neuronal recordings.
| - An equivalent circuit shows that a neuronal interface to a CMOS amplifier should not require complex d.c. bias circuitry. |
3 The Thesis

As part of the above collaborative effort [113], it was found that porous silicon (pSi) can form a biocompatible substrate to which cells seem to adhere well. It was concluded in [181], [200] and [201] that nanoporous media might form good substrates for CMOS electrodes. Unfortunately, access to the silicon substrate on standard CMOS technology is only possible by etching through the metallisation. As discussed in Section 2.9, this exposes the active regions to the physiological medium which will then cause rapid degradation of the circuits due to ionic contamination [150]. Of particular note was the observation that an adhesion molecule was unnecessary for a tight seal and that the preferential adhesion was due to the porous topography of the silicon [144]. In [202], the use of pSi in fabricated MEA electrodes confirmed that the nanoporous electrode surface was more biocompatible than a smooth surface, illustrated by the increased growth of neurites and the reduced adhesion of astrocytes (glial cells). It was therefore considered if preferential adhesion could be formed, not from the underlying silicon, but from anodising the aluminium to form a porous alumina layer. This could have the advantage of providing a porous topography similar to that of the pSi, combined with the benefit of converting the electrochemically active aluminium metal into bioinert alumina. In the context of an electrode material for biological interfaces, a review of the literature suggested that such a use of porous alumina had not been previously investigated. A porous alumina membrane has been used as a substrate for culture of neuron cells, but the membrane did not form a part of the active electrode area and the alumina had been coated with poly-L-lysine [203]. This work was also only a qualitative assessment of adhesion to the nanoporous substrate.

Research into biological interfaces using porous alumina has primarily focussed on improving bone implants. With the intention of providing a porous alumina coating to a metal implant, a commercial porous alumina membrane was shown to be biocompatible with osteoblasts cultured for 21 days [204]. Good cell adhesion was observed as the cells had a flattened morphology and had their filopodia attached to the porous alumina [205]. Other studies have

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27 ‘nanoporous’ representing pore sizes of less than 1 µm.
28 cell protrusions
shown evidence that filopodia of osteoblast and hepatoma cells interact with individual pores, with ends that can extend into the pores of sufficient size (e.g. 200 nm or 260 nm) [206],[207]. Further, it was shown that there was a difference between neutrophil behaviour between pore pitches of 20 nm and 200 nm – the cells adhering preferentially to the 200 nm substrate [208].

A quantitative study for phaeochromocytoma (PC12) cells cultured on gold sputter-coated nanoporous membranes has been conducted, showing cell density (viability) slightly increased on the porous substrate, although neurite density per cell was reduced [209]. A qualitative study including HEK293 neuronal cells, rat cortex neurons and locust ganglia showed they adhere well to uncoated porous alumina membrane with pore sizes of 30 to 200 nm [210].

From the above, and in particular [181], [207] and [208], it can be observed that the larger pore pitches of approximately 200 to 250 nm may show a positive effect on cell biocompatibility and adhesion; smaller pore sizes, and ultimately extrapolating to the zero pore size of a planar surface, may be too small to show a positive effect. This premise was carried forward to the design of experiments. Ultimately, different cell types (e.g. osteoblasts and neurons) and specific cell-lines (e.g. B50 and NG108-15) may respond very differently to their environmental factors such as substrate morphology and chemistry (See Section 2.3). However, the literature outlined above suggests that neuronal cells may grow preferentially on a nanoporous versus a planar substrate. This was the premise for the initial experiments performed as part of this work. The quantitative comparison of neuronal cell vitality for uncoated nanoporous and planar alumina is new.

### 3.1 Appraisal of Non-Photolithographic CMOS Post-Processing

The intention was to define and develop a process that would adapt CMOS for the proposed biological applications without using photolithography or other high-cost specialist equipment

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29 liver carcinoma  
30 white blood cell  
31 Pore pitch is the distance between the centres of adjacent pores, i.e. the inter-pore distance.  
32 The authors speculated that the difference in adhesion was related to the higher level of hydration of the 200 nm cells – i.e. less Al₂O₃ surface.  
33 A cell line derived from rat adrenal medulla  
34 A cell line from human embryonic kidney. Cells have many characteristics of immature neurons.
Various potential processes were devised and appraised as summarised by Figure 29 (alongside the plain porous alumina electrode (Figure 29c) already mentioned above). The following summaries relate to the figure:

a. The starting material is the unmodified CMOS metal pad (Figure 29a) with its native alumina.

b. The thickness of the native alumina may be increased by anodisation to form a barrier-type anodic layer (Figure 29b). A benefit is improved corrosion protection. However, the oxide is a good electrical insulator and would need modification to perform well as an electrode. This could be achieved by introducing leakage paths through crystal defect sites formed by electrolyte species during anodisation or by ion implantation.

c. Anodisation in an electrolyte that causes slow dissolution of the aluminium produces the porous alumina morphology (Figure 29c) that will be discussed in the following section.

d. Titanium provides a surface with excellent biocompatibility and cell adhesion characteristics [91],[211]. It may be possible to expose the titanium (or titanium nitride) barrier layer of a typical CMOS metallisation stack by selective removal of the aluminium (Figure 29d). This could possibly be achieved by a selective etch (e.g. phosphoric acid) or by first anodising the aluminium and a subsequent alumina-selective etch (e.g. sodium hydroxide). However, with an average titanium thickness of only 40 nm, it is anticipated that the electrode would have large voids and may comprise largely of a native oxide that would readily form on the titanium surface.

e. Due to titanium’s electronegativity with respect to hydrogen, it cannot be easily electrodeposited to form a layer on top of the aluminium. However, recent developments have explored electrodeposition of a monolayer formed from TiCl₄ [212]; deposition of titanium oxide has also been achieved using electrophoretic coating. But these methods would probably require high-temperature sintering and further processing to produce a conductive electrode (Figure 29e) [213],[214].
Figure 29. Envisaged design concepts for a biocompatible CMOS electrode. Aluminium thickness assumed to be 1 µm.
f. Electro- or electroless-deposition of a noble metal such as gold or platinum could be deposited directly onto the aluminium (Figure 29f). This is explored further in Section 3.2.3, but has a major disadvantage of introducing the risk of rapid galvanic corrosion of the aluminium.

g. Nanoporous substrates other than alumina may be formed indirectly by depositing thin layers onto a porous alumina surface (Figure 29g). Examples include gold, platinum, or titanium, as described above.

h. Research has demonstrated that metals can be deposited into a porous alumina film [215] (Figure 27h) but it has not been demonstrated that this is achievable on a manufacturing scale, i.e. uniformly across an array of microelectrodes. If achievable, such a layer may overcome the corrosion hazard of (f).

i. If the filling of pores with metal is practicable, then such a metal layer could readily accept a further layer of platinum black to lower the electrode impedance (Figure 29i).

j. Cellular adhesion molecules could be incorporated within the alumina pores as observed by [208], possibly without increasing the cleft size (Figure 29j). However, little work has been done that evaluates the practicality or effectiveness of such an approach.

k. The surface may be coated with a self-assembled monolayer (SAM) by silanisation (Figure 29k) [178],[216],[217]. This also enables adherent coatings of larger biomolecules to enhance cell adhesion. However, as for all biomolecule coatings, the cleft would be increased which is likely to result in poor electrical coupling.

From the above appraisal, solutions c and g-i were considered good candidates for a practical electrode. The feasibility of a CMOS porous alumina electrode will therefore now be discussed, followed by an examination of plating noble metals on aluminium.

3.2 A Porous Alumina Electrode

The natural oxide film on aluminium can be enhanced through the electrochemical process of anodisation. Two types of film can be produced, either a planar ‘barrier’ type film or a ‘porous’ type film – the type being dependent on the electrolyte used: barrier oxides are formed by anodising using electrolytes in which alumina is insoluble; porous layers are formed using electrolytes in which alumina is moderately soluble – most commonly sulphuric, phosphoric and oxalic acids.
3.2.1 Anodisation Theory

Barrier layers are most frequently characterised by their final thickness, as this parameter is precisely determined by the applied anodising voltage. Characterisations show that the thickness is dependent on the applied voltage with a linear relationship of approximately 1.0 to 1.4 nm per volt [218],[219].

![Image](image1.png)

Figure 30. Section of porous alumina. Each pore is contained within a hexagonal section of alumina wall. An alumina ‘barrier’ layer forms a hemispherical base to each pore, below which is the un-anodised aluminium.

![Image](image2.png)

Figure 31. Relationship between pore cell size (pitch) and anodising voltage for various electrolytes: 15% sulphuric acid, 10°C (1); 2% oxalic acid, 25°C (2); 3% chromic acid, 50°C (3); 4% phosphoric acid, 25°C (4). From [218].

Porous layers (Figure 30) are formed by the combined actions of film growth and the simultaneous dissolution by the electrolyte. The spacing between pore centres – the cell size – is proportional to the applied anodising voltage and is also dependent on the electrolyte type (Figure 31). For phosphoric acid the ratio of cell size to voltage is approximately 2.5 nm·V⁻¹ [218],[220],[221]. For clarity in this work, the term ‘pore pitch’ will be used henceforth instead of ‘cell size’ so to differentiate between cells of porous alumina and biological cells.

The generally accepted model describing the formation of the regular pore spacing is that current clusters at defects and at thinner areas of the native/barrier oxide. These localised areas of high current density interact with the applied electric field to influence the creation of additional oxide. This creates the trough-shaped base of each pore [219],[220]. This initial stage continues to grow a barrier type layer to a thickness that is dependent on applied
voltage. Once the trough spacing becomes stable during the anodising process, film formation proceeds by continued oxidation of the metal and dissolution of the oxide. This results in the porous layer growing downwards by consuming the aluminium layer below. The formation of the oxide relies on the transfer of $\text{O}^{2-}$, $\text{OH}^-$ and $\text{H}^+$ ions across the barrier oxide and is governed by the overall electrochemical reaction shown in (11) – more recent work providing much evidence to substantiate this model [222],[223].

$$2\text{Al} + 3\text{H}_2\text{O} \rightarrow \text{Al}_2\text{O}_3 + 6\text{H}^+ + 6\text{e} \quad (11)$$

Once a steady state is reached after initial formation of the pores, the growth rate of the porous layer is constant and its thickness is determined solely by anodisation time. Thick films (i.e. > $100 \, \mu\text{m}$) can therefore be produced, limited only by the gradual pore-widening at the top of the film which is caused by the slow dissolution of the alumina in the electrolyte. Almost infinitely variable porous layer proportions can be achieved through specification of electrolyte, voltage and a post-anodisation pore-widening etch.

### 3.2.2 Biocompatible Electrode Design

The proposed electrode design is shown in Figure 32. The key features of the design are the low impedance of the pore filled with physiological medium, the moderate impedance at the pore base and the very high impedance of the CMOS gate input.

![Model of porous alumina electrode design showing distributed circuit elements.](image)

Figure 32. Model of porous alumina electrode design showing distributed circuit elements.
Electrical models for the seal between the cell membrane and the electrode have been developed [117],[118],[120]. The cell membrane impedance is $c_m$ and $r_m$; the seal resistance, $r_s$, represents the lateral leakage path from the extracellular space below the cell membrane to the grounded electrolyte bath (Maximising this resistance is critical to forming a good electrical junction between cell and electrode); the pore resistance, $r_p$, represents the physiological medium in the pore and, due to the low resistivity of the saline medium, $r_p$ is small even for long pores up to the full 1 µm of anodised aluminium.

At the base of each pore there is a solid-solution interface. This forms a double layer as described in Section 2.3.1 and is represented here by elements $c_d$ and $r_d$. The barrier layer at the base of each pore will contribute an impedance, most efficiently represented with the $c_b$ and $r_b'$ network shown [224]-[226]. Where the entire aluminium layer has been anodised it is expected that a low impedance path, $r_b$, will be formed. This is critical to the design and is explored further in Section 3.2.2.1. Should any aluminium remain below the pore, the impedance is most likely to be that of a typical barrier layer and is represented by $c_b$ and $r_b'$.

### 3.2.2.1 Barrier Conductance

The proposed electrode design relies on minimising the impedance due to $c_b$, $r_b$ and $r_b'$ which exists at the base of each pore. Firstly, it is important to note that for a porous type film in an electrolyte it has been shown that only the barrier layer influences the film impedance [225]. Table 5 illustrates that for a.c. components of an action potential signal, an unmodified barrier layer may provide sufficient coupling to a MOS (metal oxide semiconductor) gate; at d.c. the unmodified electrode impedance may already be sufficiently low to drive a MOS gate without additional bias circuitry [113] (c.f. electrical model discussed in Section 2.5.2).
Table 5. Comparison of anodic barrier and MOS gate impedances. The source data for unmodified barrier oxide impedance is for unit area (Ωcm²). These data have been used to calculate the barrier oxide impedance for a 30 μm diameter electrode. The CMOS gate impedances are derived from gate capacitances using $Z = (2\pi f C)^{-1}$.

<table>
<thead>
<tr>
<th></th>
<th>Unmodified barrier oxide impedance – unit area</th>
<th>Unmodified barrier oxide impedance – 30 μm diameter electrode</th>
<th>Typical MOS gate impedance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.c.</td>
<td>$10^7$ Ωcm²</td>
<td>$10^9$ Ω</td>
<td>$10^{12}$ Ω</td>
<td>[224], [227], [228]</td>
</tr>
<tr>
<td>d.c.</td>
<td>$10^8$ Ωcm²</td>
<td>$10^{11}$ Ω</td>
<td>$&gt;10^{15}$ Ω</td>
<td>[229]–[232]</td>
</tr>
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</table>

However, the above barrier oxide characteristics are expected to change after complete anodisation of a thin film due to the effect of consuming the metal. Firstly, it has been shown that in the final stages of anodising that the characteristic hemispherical pore base is deformed (Figure 33) [233]. Secondly, it might be possible to produce a low conductivity ohmic contact through the barrier layer by either providing a secondary species in the anodising solution, or by coating via a post-anodisation electrodeposition. For example, it has been shown that simply by coating with silver, copper or cobalt, the barrier layer can be bridged as a result of ionic diffusion [234]. Thirdly, it should be feasible to thin the barrier layer to improve electrode conductivity. This may be accomplished either by a post anodisation etch or by electrochemical thinning based on a galvanostatic anodisation [235]–[237].

The titanium barrier layer, together with any residual aluminium, forms a conductor to the periphery of the electrode, $r_t$ (See Figure 32). The electrode is then connected to the integrated circuitry, e.g. an electroneurogram (ENG) amplifier, through the standard CMOS metallisation (i.e. the aluminium), which is excluded from the above model due to its negligible impedance.

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35 characteristic frequency of an action potential
3.2.3 Plating

It is possible to modify an aluminium electrode by plating. This may be attempted by directly plating onto the metal surface or, after anodisation, into porous alumina as outlined in Section 3.1 (the purpose being to reduce the impedance of the barrier oxide at the base of the film). Most commonly, plating with a noble metal has been used to produce a bio-inert interface. This allows a range of surfaces to be produced, suited not only to neuronal recording applications but also to many of the other biosensor applications described in Section 1.3. Unfortunately, common deposition techniques such as sputtering, e-beam and chemical vapour deposition cannot be used within the constraints of low-cost post-processing as these would require lithographic patterning of the metal to reform the pad areas. However, electroless- and electro-deposition both meet the low-cost requirement as the depositions...
would be self-patterned: the deposition being restricted to the aluminium pads exposed to the plating bath. Indeed, the apparatus is similar to that used for anodisation except that depositions are cathodic rather than anodic. Additionally, as electrodeposition requires only low voltages and currents, the bias circuits could readily be implemented as part of a CMOS biosensor circuit.

### 3.2.3.1 Electroless Plating

A CMOS post-processing technique, whereby the pads are coated with gold using an autocatalytic (electroless) gold deposition process, has already been investigated [238] – the key significance being that it is a non-lithographic process. The technique was adapted from a low cost bumping method for flip chip assembly [239],[240]. The process has been more fully characterised for flip chip assembly in [241]. Whilst flip chips require a very low resistivity contact with excellent reliability performance, the same is not necessarily true for electrophysiological electrodes: the process used by [238], depositing gold directly on top of the native aluminium oxide, avoids the wet etch and cleaning steps of [239]. The MEA used was direct coupled to the gate of a FET (part of a pre-amplifier circuit) but little electrical characterisation of the interface was performed. The work was latterly extended in [242] where electroless gold plated CMOS pads were used successfully in a bioimpedance imaging system. Here a more conventional bump deposition process was used (including a native oxide etch, zincation, nickel and gold plating steps) based on a proprietary system supplied by Shipley (now Rohm and Haas). Whilst the deposition was characterised, including failure analysis, little information was provided regarding the electrical performance of the MEA. Recently, carbon-walled nanotubes have been explored as a candidate for robust sensor materials [243]: palladium particles were used as a seeding catalyst to replace the multi-step zincation process (see above). This enabled a subsequent electroless deposition of gold that reduced the contact resistance between the single-walled carbon nanotubes and pads. However, the electroless gold plating coverage on the aluminium was poor (Figure 34), leaving exposed aluminium below and, of more concern, also leaving exposed aluminium-gold interfaces which are prone to rapid galvanic corrosion (See Section 5.5).
3.2.3.2 Electrodeposition

The fundamentals of electrodeposition are described in [244]. With respect to the electrode design, the main issues are likely to be corrosion, porosity and adhesion of the plating. However, the interfacing of reactive aluminium with a noble metal introduces a new risk: whilst an impervious layer of noble metal will prevent corrosion, any defects in the plating may allow the physiological medium to cause rapid galvanic corrosion at the interface between the metals. Such defects are common and frequently take the form of pinholes. This problem can be addressed by using an ‘underplate’ or by ensuring the plate is of sufficient thickness to eliminate all pinholes.

Underplating is usually of copper or nickel and such processes have matured [218]. Leaching of toxic underplate species into the physiological medium would require further investigation. Processing is relatively simple, such as a 15–30 s immersion of the electrode in nickel chloride dissolved in hydrochloric acid, or a three minute immersion in a solution of zinc dissolved in sodium hydroxide.

Planar gold and platinum provide bioinert surfaces for cell culture. However, it is conceivable that the surfaces may be transformed by various methods to further enhance performance. Firstly, nanoporous gold may be formed by the selective dissolution of alloying elements [245],[246]. Secondly, ‘platinisation’ produces a deposition of greatly enhanced surface area called ‘platinum black’ [247],[248]. Biocompatibility studies of platinum black have shown it to be suitable for use in-vitro and for both acute and chronic animal experiments [249]. Lastly,
porous platinum layers have also been produced by a low-cost growth method using a solution of ethylene glycol and HCl at 100°C [250].

Platinum deposition can be performed with various families of plating bath, such as sulphato-dinitrito platinous acid (Pt ‘DNS’, $\text{H}_2\text{Pt(NO}_2)_2\text{SO}_4$) at room temperature and a current of only 0.5 A·dm$^{-2}$ [251]. It may then be platinised using chloro-platinic acid (‘CPA’, $\text{H}_2\text{PtCl}_6\cdot6\text{H}_2\text{O}$) to increase its effective surface area [252].

Plating onto aluminium usually requires special treatment to remove the native oxide that may otherwise cause adhesion problems. This may be of limited concern in the context of CMOS electrode pads, as demonstrated by [238], but nonetheless can be simply addressed by various pre-treatments prior to deposition: processes include ‘pickling’ in acid to roughen the surface; anodisation, where a porous layer serves as a ‘key’ for adhesion; underplates in the form of electroless nickel or a ‘displacement film’ where the native oxide is displaced by a metal film such as zinc [218].

Plating therefore presents a flexible approach for modification of aluminium and porous alumina electrodes. It is anticipated there is considerable scope for developing new plating methods for CMOS that may produce electrochemically stable and biocompatible electrodes.

### 3.2.4 Other Electrode Design Considerations

Whilst it was anticipated that the porous alumina electrode might increase cell vitality and/or adhesion, it was unclear whether adhesion would be preferential to the electrode site versus the IC passivation. It was therefore considered whether the performance of an MEA (e.g. proportion of successful recording sites) could be increased using other supplemental techniques:

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36 Other methods rely on the complex producing binary alloys, followed by selected dissolution.  
37 Neuronal recordings have been demonstrated using platinum electrodes, as performed using the shifted electrode (photolithographic) method on CMOS, developed by the Physical Electronics Laboratory, ETH, Zurich [160].
i. D.C. electrode bias: A hypothesis of the Bath/KCL collaborative group was that a small d.c. bias on the electrode pads may influence the adhesion of cells. An experiment was devised to explore this and is outlined in Appendix A. No effect was noted.

ii. Electrophoresis: This established method of moving colloids using an electric field within an electrolyte is outlined in Section 2.7. In the form of negative dielectrophoresis (n-DEP) the technique has been successfully developed to increase the proportion of cells above electrode sites but it probably does not improve adhesion [146],[189],[253],[254].

iii. Cell plating density: The number of functioning MEA recording sites improves with cell density: this is a key factor as to why cortical tissue slices presently give better MEA performance than cultured neurons. It may therefore be possible to increase MEA performance by simply increasing the duration for which the cells grow on the MEA: this may be achieved by increasing the time that the cells are in growth medium within the culture chamber, prior to changing to plating medium [255]. A disadvantage is the increased frequency of culture medium changes needed to maintain the vitality of the cells and an increased risk of contact inhibition.\[38\]

Since d.c. bias showed no effect and electrophoresis does not necessarily improve cell-substrate adhesion, these techniques were not pursued in the body of this research as they were unlikely to improve directly the neuronal recording success rate using CMOS pads. Increasing the cell plating density may improve the probability of a cell covering an electrode, but it was considered unlikely that this factor alone was the cause of the 0% success rate. Plating density was however considered important as a secondary parameter during all biological experiments in this research. Cell culture protocols were frequently optimised to maximise the cell density (as detailed within experimental sections of this work).

It was also considered that the general approach of using anodisation to overcome the limitations of aluminium CMOS electrodes may form a foundation upon which many variants of electrode could be developed. The appraisal of Section 3.1 proposed several enhancements to the basic porous alumina electrode, but this list is far from comprehensive. For example, it might be possible to deposit (porous) platinum black directly into the alumina pores without

\[38\] The contact between two or more cells inhibits further growth. This may also result in cells detaching from a substrate and dying.
the intermediate step of depositing a (compact) metal layer. This might result in the desired lower impedance yet retain the benefits of a porous substrate. Alternatively, it might be feasible to partially fill the pores with a noble metal and then etch away the alumina walls to leave an electrode of metal nano-rods with high surface area [256]. This might have low impedance comparable to the dendritic titanium nitride electrodes of the Multi Channel Systems MEAs.

3.3 Patent Filing
The porous alumina electrode discussed in Section 3.2 is a novel approach. The author, as lead inventor, together with three co-inventors, has filed a patent application (‘Biocompatible Electrode’, UK patent GB0820629.4 and international patent PCT/GB2009/002641). The international search report performed recently by the European Patent Office showed the claims relating to CMOS processing are likely to be considered novel and comprise inventive steps. The invention was a direct result of this PhD research. An excerpt of the filed application is attached as Appendix M.

3.4 Scope of the Thesis
Since defining this thesis, the value of the research topic, including confirmation that the electrode interface is the present roadblock to CMOS biosensor commercialisation, has been corroborated by [199] where they state,

‘The primary design challenge using CMOS technology is the interface design between assay and integrated chip (IC) which generally calls for additional post-fabrication steps to facilitate compatibility in detecting targets (e.g. analytes).’

It cannot be over-emphasised that the intention of the work was to investigate the development of biocompatible electrodes using only standard CMOS devices. Only by minimising cost can the devices be used for high volume applications (e.g. high throughput screening) or be made economic for single use (e.g. electrophysiology recordings). It is acknowledged there has been much progress outside of this cost constraint. For example, dendritic titanium nitride electrodes have been demonstrated to perform well as low impedance microelectrodes [194], but titanium nitride is not accessible on standard CMOS for reasons explained in Section 2.8. It is acknowledged that titanium nitride can be deposited and
patterned on top of a CMOS process but this would require photolithography within a fabrication plant and so puts the technique outside the scope of this work. Other similar claims for ‘standard CMOS’ biosensors exist but these also require photolithographic processing [49] or use layers present in CMOS but are out of sequence\textsuperscript{39} with all known functional IC processes [271].

For the same reason as explained above, many other fields are excluded. MEMS and BioMEMS incorporating micro-fluidics are an extremely useful basis for developing biosensors but are not achievable with standard CMOS [257],[258]. Biosensors based on thin-film transistors (TFTs) have the appeal of being able to incorporate flexible polymer or a variety of rigid substrates other than silicon [259] but are not conducive to the very large scale integration (VLSI) required for on-chip signal and data processing.

The integration of amplifier and logic functions onto the CMOS IC is required for the technology to add value. However, many sophisticated IC designs for neuronal recordings have already been demonstrated (Section 2.9.2) and so the circuit design aspect is not a focus of this thesis: in fact the CMOS MEA used in previous work at The University of Bath and King’s College London was a CMOS device with absolutely no transistors or other electronic components. This approach has been carried forward since the direct connection between pads and package pins is most suited to electrode development and characterisation.

The emphasis of the research is a cell-based biosensor for neuronal recordings. Due to the small signals, this is an extremely demanding electrode to design and therefore, if successful, it should also be capable of being configured for less challenging functions (e.g. neuronal stimulation, ECIS, electrophoresis or as a transducer incorporating a DNA, enzyme or antibody bioreceptor). Indeed, it was felt that the flexibility of the approach could be illustrated by extending the scope to include one other such application: ECIS is explored in Chapter 11.

The central and most direct contribution of the research is therefore in the field of biosensors. Related topics are biomedical engineering, bio-electronics, lab-on-a-chip, electrophysiology

\textsuperscript{39} A CMOS process is not only defined by its different layers but also by the sequence in which each layer is deposited or grown. For example, metal layers are never found below MOS gate (oxide and semiconductor) structures.
and neuro-prostheses. Related fields include drug discovery, electrochemical sensors and bio-separation, but also extend to environmental protection, food safety, defence and security. The novel IC packaging is relevant to MEMS generally and other IC-based biosensors. The biocompatibility and adhesion work relates to neuroscience, toxicology and cell biology. Other novel aspects of the research contribute to materials science and electrochemistry (i.e. anodisation and electrodeposition) and are within the scope of nanotechnology. The CMOS basis makes the research of peripheral interest to the semiconductor industry (process technology and IC design).

3.4.1 The Low Cost Criterion

It is stated above that the scope of the thesis is limited to exploring only ‘low cost’ post-processing methods. For the purpose of this work, this term will be defined by the following: Firstly, a product cost after post-processing that is comparable to one incorporating a standard (unmodified) CMOS IC [151]. Secondly, a manufacturing cost that enables a product to be priced competitively versus a similar product manufactured using the alternative post-processing methods (such as the ‘shifted electrode’ process of Figure 25).

From a commercial viewpoint, a more thorough understanding of projected production costs must be performed and a business risk assessment made against competing technologies. For example, the proposed ‘bench top processing’ required to create the porous alumina biocompatible ICs seems intrinsically cost effective, but rigorous business analysis is required after scaling to production line volumes [260]. However, the premise of this work can be considered sound, as long as it is accepted that the cost of labour and the few stock solutions required for the porous alumina electrode will be more cost-effective than the installation of additional stations in a semiconductor fabrication plant that are required for the ‘shifted electrode’ approach. Further, it should be noted that such customised processing stations are not generally allowed into a high volume production facility as this can jeopardise production yields [261].

Throughout the period of this research, the evaluation of ‘low cost’ remained necessarily subjective: a quantitative evaluation of product cost can be made only once a target application has been chosen and the product design specification completed. For example, the manufacturing costs of a simple biocompatible CMOS MEA designed for electrophysiology
The research will be different to a microtitre plate incorporating an IC in the base of each of the 384 wells. Pricing and acceptable manufacturing costs will also be defined by what the target market(s) will tolerate. This issue is re-iterated in the discussion of future work (Section 12.1).

3.5 Summary

A review of the literature has shown that passive MEAs form the basis of present commercial markets since the promised benefits of CMOS cost-effectiveness and higher spatial resolution are being held back by problems with biocompatibility of the electrode. The performance of an electrode is based on good cell adhesion which is influenced by its surface chemistry and morphology. The problems have been illustrated by previous work performed by Taylor et al. where the recording success rate from CMOS electrodes was 0% compared to the 5.6–18.5% of commercial titanium nitride electrodes.

The aim of this research was to improve the performance of CMOS electrodes with the intent that the technology’s benefits may be realised in a commercial context. This is measured by:

i. designing an electrochemically and electronically stable CMOS electrode

ii. ensuring cell vitality through good biocompatibility

iii. demonstrating recording of action potentials

iv. working within the low-cost processing constraint

The literature suggests that nanoporous alumina may promote good adhesion in the same manner that has been shown for neuronal cells on porous silicon and for osteoblasts on porous alumina. Additionally, the literature suggests that alumina should provide a biocompatible and electrochemically stable surface for cell culture.

The originality of the research is in the design of a CMOS electrode that meets the low-cost criterion. The conversion of CMOS metallisation to form a porous alumina electrode is novel and should meet the biocompatibility and electrochemical stability prerequisites. It is likely that anodisation of CMOS has been overlooked or unduly disregarded since alumina makes a very poor electrode in its unmodified form. However, the adoption and customisation of techniques recently published may be used to enhance the electrical characteristics of porous alumina.
The remaining sections focus on the experimental work in which CMOS electrodes based on porous alumina are developed, characterised and validated by demonstration.

Key Points

- Development of the electrode interface is the primary challenge in using CMOS in cell-based biosensors
- Published methods do not fulfil a low-cost CMOS post-processing criterion
- Conversion of aluminium CMOS pads into porous alumina has the potential to meet the criteria of low-cost processing, electrochemical stability, biocompatibility and good cell-substrate adhesion.
- A porous alumina electrode may form a foundation for specific electrode designs that are optimised for various biological applications
- Patent office searches support the claims of novelty made in the thesis
4 Overview of Experimental Investigations

The overall objective of the experimental work was to investigate whether a porous alumina electrode based on CMOS could overcome the failings of unmodified CMOS aluminium pads. However, at the outset of the work it had not even been demonstrated that aluminium CMOS tracks could be anodised: it was unclear whether the current density would be too high and fuse the aluminium tracks; the anodising electrolyte might destroy the chip passivation. It might not be possible to convert the electrically-insulating porous alumina into a conducting electrode. Even if the electrical characteristics of the electrode were correct, it remained unclear whether the porous alumina would be biocompatible and whether neuronal cells would adhere to it. Then there would remain the ultimate test of whether the electrode would successfully record action potentials.

A difficulty at the outset of this work was funding. CMOS is low-cost when manufactured in volume but prototyping is relatively expensive (~£7k for 30 devices with die no larger than 10 mm²). The total area of a substrate required for several runs of a cell vitality experiment is large (~10⁴ mm²) compared to the small combined area of microelectrode (~10⁻² mm²) available on a single 10 mm² chip. So another hurdle was whether these tests could be performed without using excessive numbers of expensive ICs. It transpired that metal deposited on glass coverslips would adequately emulate CMOS metallisation and so coverslips were used for much of the anodisation and cell biocompatibility experiments. A further difficulty was how to analyse the nanoporous films: it is not possible to see nanoporous alumina using either the naked eye or optical microscopes and the SEM facilities at the University of Bath were eventually to prove inadequate. Effort was required to secure additional funding to use a high resolution field-emission SEM and focussed-ion beam (FIB) at Cardiff University. Such difficulties are possibly not bourn out by the body of this thesis, but access to facilities and equipment was to require considerable administration.

The experiment chapters therefore begin by exploring the basic capabilities (Chapter 5) of anodising thin aluminium films to produce porous alumina and testing its biocompatibility with neuronal cells. This chapter also encompasses evaluation of corrosion in biological media and modelling the electrical characteristics at the bases of the pores (across the barrier
oxide). This work, for the first time, sets out the principles for a porous alumina electrode (Appendix H).

Due to reasons that become evident through the work of Chapter 5, the development of new techniques were required to study the effect of substrate morphology on cell adhesion and is the basis for Chapter 6. A discussion of differences between cell vitality and adhesion, together with a new form of adhesion assay, were published in a second journal article (Appendix I).

Focus then returns in Chapter 8 to the electrical performance of the electrode. It was postulated in Sections 3.2.2 and 3.2.2.1 that the electrically-insulating porous alumina could be modified to produce a conducting electrode and that achieving this was a critical success factor. Therefore, as a prerequisite to studying barrier impedance, it was necessary to develop a novel method for measuring impedance in real-time during electrode fabrication. Secondly, it would become necessary to demonstrate that development work performed using coverslips could be translated onto CMOS ICs. At this point attention is therefore given to the design of the ICs (Chapter 7). The ICs also require packaging prior to use and this section also investigates two new prototype assembly methods, leading to a third journal publication (Appendix K).

Two approaches were taken forward for further research (Chapter 9). Firstly, the plain porous alumina electrode was explored, with modification of the barrier oxide to lower its impedance. Secondly, the deposition of a noble metal into the pores was investigated, which also relies on lowering the barrier oxide impedance. This work utilised both coverslips and CMOS ICs. The results were published as a fourth journal article (Appendix J).

The last thrust of the experimental work was to evaluate the electrodes developed in Chapter 9 by demonstrating their use for neuronal recordings (Chapter 10) and for ECIS (Chapter 11).

All experiment work was carried out solely by the author, with the exception of SEM imaging, the dissection of rats as a supply dissociated neurons, the fabrication of the mould template used for IC packaging and the occasional cover for routine cell culture tasks at King’s College London.
5 Preliminary Materials and Biocompatibility Evaluations

5.1 Introduction

The proposed porous alumina electrode design was investigated. The objectives of the experiments were to:

i. establish basic anodisation and physical analysis capability
ii. evaluate basic electrical characteristics during anodisation
iii. determine if anodisation is suitable for implementation in CMOS
iv. evaluate the biocompatibility of porous alumina for neuronal cells
v. characterise adhesion versus alumina pore pitch

5.2 Anodisation Experiments

5.2.1 Introduction

The anodisation process is fundamental to the proposed electrode design. These initial experiments therefore established the necessary resources for this work and studied the anodising characteristics specific to thin films.

5.2.2 Materials and Methods

A limited number of CMOS passive MEA chips assembled for the previous work of The University of Bath and King’s College London [113] were available for further experiments. However, the small combined area of the electrodes per chip provided limited opportunity for evaluating anodisation methods, biocompatibility and cell adhesion. For this work it was therefore decided to reproduce the aluminium electrode surface on glass substrates.

It has been observed that since adherent cells settle under the influence of gravity, surfaces raised up within the culture medium, such as the top of a standard microscope slide ~1.2 mm above the dish base, may suffer from a reduced cell count. Therefore, it was decided to work with glass microscope coverslips which have a thickness of only 200 µm.
Coverslips were coated to reproduce closely the metallisation of the CMOS passive MEAs fabricated by austriamicrosystems AG on their 0.8 µm process (Figure 23). This is a two layer metal process with electrodes formed by opening windows in the passivation onto Metal2 (the upper metal layer), below which is always a glass interlayer dielectric (ILD)\textsuperscript{40}. The Metal2 stack was reproduced by depositing ~40 nm of titanium onto the coverslip followed by approximately 960 nm of aluminium (Teer Coatings Ltd, UK). It should be noted that titanium nitride anti-reflective coatings used on other processes are removed from the pad areas when etching the passivation and therefore the coated coverslips are representative of these processes also [262].

The incorporation of the 0.5 wt% copper and 1.0 wt% silicon was considered: it has been shown that for an Al-Cu alloy with a low concentration of copper (<1%), anodisation proceeds on a similar basis as for pure aluminium, except for a copper-enriched layer gathering at the metal-alumina interface, depletion of copper within the alumina and a slightly slower rate of film growth [263]–[265]. At this stage in the work, it was expected that the silicon would result in immobile cations within the alumina and likely to have only minimal effects on the porous layer formation [222]. For these initial experiments it was therefore decided to deposit pure aluminium (99.9%) onto the coverslips without the alloying elements. It should be noted, however, that the effects of the alloying elements were expected to play a critical role in the electrical characteristics of the electrode (Section 3.2.2.1) and are investigated later (Section 9).

Additional coverslips were coated in-house using a BOC Edwards 306A Thermal Evaporator to a thickness of approximately 1.0 µm of aluminium without the Ti barrier layer. These were used for equipment calibration and basic anodisation tests where the Ti barrier was of no consequence. The porous alumina morphologies chosen for investigation were guided by the literature as outlined in Section 3, which coincidentally corresponded to the maximum attainable pore pitch using the available apparatus: by choosing a phosphoric acid electrolyte and working up to the maximum voltage of 100 V from the supply, the theoretical pitch of 250 nm could be achieved.

\textsuperscript{40} The only location where ILD is not below Metal2 is in ‘vias’ (connections) down to Metal1. It will be discussed later how the placement of such vias under the electrode pads precluded these early CMOS MEA chips from being used for later experiments.
For these preliminary experiments, the basic apparatus was an open glass beaker containing approximately 200 ml of electrolyte, magnetic stirrer, thermometer, platinum gauze cathode and the coated coverslip suspended using a miniature crocodile clip (Figure 35). Bias was provided by a Keithley 236 Source-Measure Unit operated by a PC running a custom Labview 7.1 (National Instruments) program. This enabled easy selection of either potentiostatic\[^{41}\] or galvanostatic\[^{42}\] anodisation, bias ramping, and datalogging of the respective current or voltage. Potentiostatic anodisation was generally used to give a pitch that is constant throughout the height of the film: galvanostatic anodisation would give a branching pore structure not conducive to a simple electrode design \[^{220}\]. Initial trials were performed using the PTFE\[^{43}\] sample holder that forms an electrical connection to the backside of the aluminium anode and seals the connection from the electrolyte using an O-ring.

A single CMOS IC (‘WET3’) from previous work at The University of Bath was used to test on-chip anodisation using the results from the work on coverslips. The IC, with 48 pads of 30 µm diameter was anodised at 40 V using a 4% phosphoric acid electrolyte in the culture chamber. This preliminary IC design was not optimised for anodisation and had metal tracks between bondpads and electrodes that were only ~2 µm wide. To avoid fusing these narrow tracks, the large initial current flow during barrier oxide formation was limited by ramping the potential to 40 V over 30 s.

\[^{41}\text{constant voltage}\]
\[^{42}\text{constant current}\]
\[^{43}\text{polytetrafluoroethylene}\]
5.2.3 Results and Discussion

Figure 36 shows the resulting datalog from an initial trial to set up the apparatus using 2 mm thick aluminium sheet. This is a typical I-t characteristic where, during an initial high current stage, the barrier layer is formed (100 mA supply compliance). After approximately 6 s the porous layer starts to form and subsequently becomes the steady state.

![Figure 35](image)

**Figure 35.** Anodisation apparatus: beaker with electrolyte, PTFE anode holder and platinum mesh electrode (a); electrode connections (b); magnetic stirrer (c); source/measure unit (d); PC running Labview (e).

![Figure 36](image)

**Figure 36.** Potentiostatic anodisation of approx 0.8 cm\(^2\) of mechanically polished aluminium sheet (unknown purity). \(V = 12\) V, 4% phosphoric acid, \(T = 25^\circ\)C.
5.2.3.1 Thin-Film Anodisation

Anodisation is generally performed using a bulk aluminium substrate that is much thicker than the anodic layer to be formed on coverslips and CMOS. The back of an aluminium thick sheet therefore provides an electrical connection of negligible resistance throughout the anodising process. However, it may be anticipated that anodisation of a thin aluminium film could result in particular physical and anodising characteristics in the porous layer relating from the final stages of consumption of the aluminium. These physical traits are explored in Section 3.2.2.1 and below. With respect to the anodising characteristics, aluminium thin films are usually on either a thick conducting (e.g. indium tin oxide) or semiconductor (e.g. silicon) substrate that provides a relatively low resistance path to the anodising aluminium. However, when anodising on a glass insulator\textsuperscript{44}, the final stages of consumption of the aluminium result in an increasing access resistance as shown in Figure 37. During period (a) the barrier layer is formed and here is limited by the supply 100 mA compliance. During period (b) the barrier layer growth is completing and the porous layer begins to form. Period (c) is steady state porous layer growth. As the pores reach the base of the aluminium film, the current falls (d). The 40 V anodisation curve shows that continuing to apply bias after the aluminium is consumed results in a steady state leakage current (e).

\textsuperscript{44} remembering that the glass substrate is representative of the insulating CMOS inter-layer dielectric
Chapter 5 – Preliminary Materials and Biocompatibility Evaluations

5.2.3.1.1 CMOS Scaling

Having established that the coverslip metallisation can be successfully anodised, it was necessary to confirm (initially by modelling) that the observed current densities would not exceed the maximum ratings of a CMOS circuit. The anodisation current is proportional to the anode area in contact with the electrolyte. Therefore the current density is independent of the surface area anodised. The relationship between two different areas is simply \( I_1/I_2 = A_1/A_2 \), where \( I_x \) are the anodising currents and \( A_x \) are the respective anodising surface areas.

With a coverslip anodising area of \( A_1 = 400 \text{ mm}^2 \), a circular CMOS electrode area of radius, \( r \), of 15 µm (\( A_2 = \pi r^2 \)) and a peak coverslip anodising current of 100 mA for \( V \leq 100 \text{ V} \), the peak CMOS current for one pad is estimated to be 177 nA. This provides excellent margin to the typical absolute maximum rating (short duration load) for an individual CMOS I/O pad of 100 mA. The anodisation current could also be sourced through the usual VDD supply for anodising an array of electrodes in parallel, assuming maximum ratings for the supply are not exceeded.

Figure 37. Typical anodisation of aluminium thin films (Oxalic acid, 40 V and 60 V, 10°C, on 40 nm Ti and glass substrate. Anodised area, \( A \approx 400 \text{ mm}^2 \); barrier forming (a); barrier completion (b); porous layer growth (c); aluminium film consumed (d); leakage current (e).
5.2.3.2 Fusing

A significant processing problem was discovered when attempting to use the suspended coverslip arrangement for anodising at the higher voltages of 80 V and 100 V. Under this arrangement, the duration of barrier layer formation (period (a) in Figure 37) is governed by the compliance\textsuperscript{45} of the supply and the actual voltage applied to the anode ramps over this period until the target potentiostatic bias is reached: this period is, in effect, galvanostatic anodisation. It was found that fusing would occur along a line formed on the coverslip by the surface of the electrolyte: this failure mode was the same in every example.

It is likely that the high current during barrier formation results in joule heating of the thin film and that when anodising at 80–100 V the duration of this heating is sufficient to cause melting of the aluminium. It was postulated that the line of fusing at the electrolyte surface is determined by the point of highest current density: given that the current density is constant across the surface area undergoing anodisation in the electrolyte, the current in the aluminium film increases linearly towards the suspended top end of the coverslip (Figure 38). Given that the film is being consumed by the anodisation process, it can be seen that the location of highest current density, $J$, is along the surface of the electrolyte.

\textsuperscript{45} The compliance of a power supply (supplying constant voltage) is the operating range over which the current is within limits, the limits being set by either the user or the equipment specifications). For example, the Keithley 236 Source-Measure Unit has a maximum current specification of 100mA. Therefore, the current output will be limited to 100mA regardless of the set voltage. This state of operation is often referred to as ‘compliance’.
An attempt was made to eliminate the fusing by increasing the rate of barrier film growth and lowering the temperature (RTE-101 Constant Temperature Bath and Circulator, ThermoNeslab Instruments, Inc.) by diluting the phosphoric acid electrolyte with 25% ethanol. This permits faster film growth without burning and enables working temperatures as low as −10ºC [266]. Since this failed to prevent fusing it was decided to submerge the entire coverslip in the electrolyte. This was accomplished by soldering a sheathed wire to the centre of the coverslip (Carrs ‘Grey Label’ Flux and Carrs ‘No. 179’ solder, 4D Modelshop Ltd) and insulating the connection using silicone sealant (Dow Corning). Cooling the electrolyte to 10ºC using the chiller and a copper coil resulted in no further fusing.
5.2.3.3 Pore Pitch Image Analysis

It was necessary to test whether the porous layer pore pitch conformed to the expected 2.5 nm·V⁻¹ anodising ratio [218]. For the study of highly-ordered porous layers, this task is simplified by the regularity of the pores. However, the unordered nature of simple anodised layers makes this task more complex. It was therefore necessary to use image analysis software to calculate the mean pitch from the SEM images. With an irregular pore structure there is no single method for determining which pores are adjacent.

Three models were considered:

1. assuming a regular square layout of pore centres, the mean inter-pore distance, \( d \), is \( \sqrt{(A/n)} \), where A is the area represented by the image being processed and n is the number of pores within that image.
2. if a hexagonal pore structure is assumed, as expected for highly-ordered films, \( d = \sqrt{(A/\sqrt{3}n)} \).
3. a linear profile of an SEM image with analysis to count the number of threshold transitions along its length.

Two software methods were evaluated:

1. ImageJ – an open-source Java-based application that enables counts of hole-type features [267].
2. PoreAnalysisSEM, an ImageJ plug-in [268]

The PoreAnalysisSEM utility was found to have difficulties in the scaling algorithms and was therefore rejected. ImageJ is a well-established open source utility: a basic check of the pore-count utility was performed using dummy images with a known number of ‘pores’ and confirmed the algorithm was reliable for high-contrast images.

<table>
<thead>
<tr>
<th>Anodising voltage (V)</th>
<th>Expected pitch, based on anodising ratio (nm)</th>
<th>( A (\mu m^2) )</th>
<th>( n )</th>
<th>( d = \sqrt{(A/n)} ) (nm)</th>
<th>( d = \sqrt{(A/\sqrt{3}n)} ) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>75</td>
<td>4.4</td>
<td>461</td>
<td>98</td>
<td>74</td>
</tr>
<tr>
<td>40</td>
<td>100</td>
<td>4.4</td>
<td>350</td>
<td>112</td>
<td>85</td>
</tr>
<tr>
<td>60</td>
<td>150</td>
<td>4.4</td>
<td>233</td>
<td>138</td>
<td>105</td>
</tr>
<tr>
<td>80</td>
<td>200</td>
<td>4.4</td>
<td>146</td>
<td>173</td>
<td>131</td>
</tr>
</tbody>
</table>

Table 6. Pitch image analysis for a selection of porous substrates.
The above models yield the pitch sizes shown in Table 6 and Figure 39. The reason for the smaller than expected observed pitches is not presently understood, but is possibly due to a combination of the following:

i. a limitation of the models, as pores do not conform to the assumed regular spacing.

ii. the difficulty in establishing the inter-pore boundaries – either by the image analysis software or manually – especially since pores branch and merge considerably along their length, particularly at the surface of the film which is formed first during anodisation.

Because of the difficulties in measuring average pitch, it was decided that a more suitable measurement might be porosity – the ratio of pore area to alumina surface area (c.f. Section 6.3).
5.2.3.4 Pore-Widening Etches

Whilst the pitch of porous alumina film increases linearly with anodising voltage, the ratio of pore area versus alumina area, i.e. the porosity, decreases (Figure 40). This is due to the dissolution rate of the pore wall being constant for all films: the dissolution is therefore more pronounced on films produced at lower voltages, and, for a given film thickness, these are anodised for longer [219]. To modify the films so they have porosity similar to the biocompatible porous silicon and alumina substrates of the work outlined in Chapter 3, the pores of the 150, 200 and 250 nm substrates were widened. The standard method of widening is a simple etch performed after anodising, using the 4% phosphoric acid [203],[210],[221], [269]–[271].

To establish the rate of pore-widening, a single substrate was cleaved into four parts, each piece being etched for increasing duration (Figure 41). The rate of wall thickness etch was analysed by comparing wall thicknesses and calculating the mean rate of dissolution. Printed images were used to manually select and hand-measure approximately 15 wall thicknesses from each specimen. The results are shown in Figure 42 and show the mean rate of
dissolution to be approximately 9 nm·min⁻¹ for the chosen conditions of 4% phosphoric acid at 45°C. These data were used in subsequent pore-widening etches for substrates of 150, 200 and 250 nm pore pitches. As future work, it may be useful to re-establish an etch rate at room temperature to avoid the need for controlled heating of the etchant.

Figure 41. Pore widening of 150 nm (60 V) substrate: a. not widened; b. etched for 2 minutes, 4% phosphoric, 45°C; c. etched for 3 minutes; d. etched for 4 minutes. All images at 50 kx magnification, as per scale bar. (Hitachi S-4300 FE-SEM.)
5.2.3.5 On-chip CMOS IC Anodisation

The single ‘WET3’ IC was anodised. SEM analysis (Joel 6310) confirmed successful anodisation (Figure 43). *This demonstrated that standard CMOS metal tracks are capable of carrying sufficient current to electrode pads undergoing anodisation and substantiates the current scaling model discussed above.*

As an aside, it was noted that the quality of the anodisation was poor due to the WET3 design not being optimised for such processing. In addition to the tracks being of minimal width (hence resulting in a higher than necessary current density), the pads were formed of a silicon ‘contact’ and Metal1 stack directly beneath the Metal2 stack. This resulted in a multi-layer sandwich of Al-Si-Cu / Ti / TiN / Al-Si-Cu / Ti / Si. Many pads were noted to have disintegrated and it was believed that the damage had been caused by the lifting off of each anodised layer as subsequent (lower) layers were reached. However, these effects were not believed to be significant as an optimised pad would not include the unwanted layers (i.e. the Metal2 stack would be designed to be above the insulating inter-layer dielectric (ILD)).

Figure 42. Graph showing pore-widening etch rate to be approximately 9 nm min⁻¹ in 4% phosphoric acid, 45°C. *(n ≥ 10 for each specimen). Error bars are 95% confidence intervals.*
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5.2.4 Conclusions

The above experiments therefore met the objectives of establishing basic anodisation and analytical capability and evaluated the basic electrical characteristics during anodisation. Most importantly, the ability to anodise true CMOS pads was demonstrated for the first time. Before extending the anodisation research further it was necessary to evaluate the biocompatibility of porous alumina with neuronal cells. This is therefore the next set of experiments to be introduced.

Figure 43. Anodising of CMOS pad: (a) assembled IC with culture chamber and exposed pad array; (b) array of 48 pads; (c) SEM image of a single pad, tilted 55°; (d) an anodised pad (30 V, 4% phosphoric acid, 22°C), with passivation at lower right.
5.3 Experiment to Assess Alumina Pore Size as a Factor for Biocompatibility

The experiment objectives were to firstly test whether porous alumina is a preferential surface for NG108-15 cells compared to aluminium native oxide and, secondly, characterise which alumina pore pitches, if any, were preferred.

5.3.1 Methods

Cell culture was performed at King's College London under the supervision of Dr J. Robbins. For evaluation of electrode biocompatibility and adhesion, the choice of NG108-15 cell line was governed by proposed applications, ease of use and past experience [113]. These mammalian neuronal cells are a hybrid between mouse neuroblastoma and rat glioma. The cells, being clonal, have the advantage of behaving similarly to a given environment and are free from satellite cells that often accompany primary neurons [272].

Cells were cultured in 50 ml flasks, each containing 9 ml of growth medium (Dulbecco’s Modified Eagle’s Medium (DMEM) with GlutaMax-II (Gibco), 5% foetal calf serum (FCS, Invitrogen), HAT supplement (30 µM hypoxanthine, 0.12 µM aminopterine, 4.8 µM thymidine, Sigma-Aldrich), 5 ml Penicillin-Streptomycin (Sigma-Aldrich) and incubated at 37°C, 10% CO₂. Passaging was performed with a 3:1 division when cells were approximately 60–70% confluent, or occasionally 2:1 when only 50–60% confluent.

5.3.1.1 Cell Vitality Protocol

Anodised coverslip substrates were cleaved into approximately six squares of area ~1.0 cm² using a diamond scribe. Each substrate piece was scribed with the letter ‘b’ on its bottom-side to provide a visual check for correct orientation throughout the experiment. Handling was kept to a minimum to prevent scratching the surface. Sterilisation of the substrates was performed in the laminar flow hood by submersing in ethanol for 30 mins followed by air drying for approximately 15 minutes. The substrates were subsequently moved to sterile 35 mm dishes (Nunc) onto which 2 ml cells were plated out and incubated at 37°C, 10% CO₂. Growth medium was replaced after 24 hours with plating medium (DMEM with GlutaMax-II, 1% FCS, HT supplement (30 µM hypoxanthine, 4.8 µM thymidine, Sigma-Aldrich), 5 ml

---

46 foetal calf serum
Penicillin-Streptomycin). The cells were then incubated for a further three or four days prior to measurements (this duration being kept consistent for all runs of a given experiment).

The neuronal characteristics of NG108-15 cells can be enhanced by differentiating for 48 hours (by the addition of isobutylmethylxanthine (IBMX, 50 µM) and prostaglandin E1 (10 µM) to the plating medium). However, it was decided that differentiation was unnecessary for the vitality experiments.

Density of cells was normalised across different runs by using plain glass coverslip sections of ~1 cm² as a control. Density of cells in each dish for a single run was regulated by using a micropipettor to dispense precisely 2 ml of cells to each dish. To ensure cells were not being damaged by handling using a micropipettor a cell count comparison was performed versus handling with a 10 ml syringe and Kwill (tube filling cannula, Fisher). No significant difference in cell count was found between the two methods and so the micropipettor was used for subsequent plating out.

### 5.3.1.2 Cell Staining

A phase-contrast inverting microscope is usually used for cell culture as this emphasises cell structure such as membrane, nuclei and extending processes (Figure 44a). However, phase-contrast microscopy relies on a transparent substrate and therefore cannot be used with aluminium-coated glass. It was therefore necessary to use a microscope with epi-illumination (SMZ1500, Nikon) but it was found that this gave insufficient contrast between the cell and the aluminium/alumina substrates (Figure 44c, e). However, it was found that sufficient contrast could be established by staining the cells: from separate work that evaluated Methylene Blue, Brilliant Blue G and Nile Red, it was found that the former was most successful [273].

Briefly, the staining protocol was to add 5 mg methylene blue to 100 ml of buffer solution (Distilled water, NaCl 120 mM, KCl 3 mM, MgCl₂ 1.2 mM, NaHCO₃ 22.6 mM, Glucose 11.1 mM, HEPES 5 mM, CaCl₂ 2.5 mM, with pH adjusted to 7.36 using HCl or/and NaOH). The plating medium was removed from each 35 mm dish and replaced with sufficient stain to cover the substrates. After a dwell time of 45–60 minutes, the substrates were lifted and placed into dishes containing plain buffer solution.
It should be noted that methylene blue does not distinguish between live and dead cells. Ideally, a vital stain would be used, but would require an epi-illuminated fluorescence microscope, which was not available.

Refinements to the methylene blue protocol included:

i. warming the buffer to ~37°C to enhance dissolving of the crystals
ii. filtering to remove residual crystals (Millipore membrane filter)
iii. removal of cells from the underside of the glass coverslips to ensure only cells on top of substrate were included in cell counts: wiping of the underside was performed using cotton buds soaked in buffer solution.

Microscopy was performed with most of the buffer temporarily removed from the dish so to improve the image quality: however, the substrates were not allowed to dry at any instant.
5.3.1.3 Cell Counts

Initially, cell counts were performed manually from digital images of the substrates. Since gridded dishes could not be used because of the opaque substrates, it was decided to perform cell counts using the entire microscope field (SMZ1500, Nikon, 11.25x zoom). A Nikon D200
10.2-megapixel camera with a 10x photo tube lens was used, resulting in a magnification of $7.31 \times 10^6$ pixels·m$^{-1}$.

However, it was desirable to automate the cell count process as subsequently more than 800 images were taken from only the initial three experiments. Therefore, during the first experiment (see below), the open source ‘Cell Profiler’ software [274] was evaluated by comparing automated and manual cell counts. The process relied on the above cell straining protocol where the blue cells could be detected by automatically filtering the images (Figure 45). The results (Figure 46) showed the discrepancy between the two methods was 14.4%, but due to difficulties in estimating number of cells within clumps, it could not be determined which method was more accurate. It was decided that this error was acceptable and that by avoiding clumps of cells the outliers and cell count errors could be minimised.

![Figure 45. Automated cell counts: a. cells are clearly identifiable after initial staining (porous alumina, '206 nm' pore pitch; scale bar is 400 µm). The white dots are remnants of aluminium metal that have not been anodised at the base of the porous alumina film. A vertical scratch at the top and a diagonal scratch at the bottom of the image were applied to this trial substrate as reference marks; b. the blue-stained cells in image (a) are emphasised by applying a red filter in the ‘Cell Profiler’ software; c. cells can then be correctly identified by ‘Cell Profiler’ image analysis.](image-url)
5.3.2 Design of the Experiment

Standard statistical tests were used [275], with null hypothesis, $H_0$, hypothesis, $H_1$, and population mean, $\mu$. The null hypothesis was that there was no difference between number of cells (vitality) on native oxide ($\mu_{\text{Al}}$) and porous alumina ($\mu_{\text{porous}}$):

\[
H_0: \mu_{\text{Al}} = \mu_{\text{porous}}
\]
\[
H_1: \mu_{\text{Al}} \neq \mu_{\text{porous}}
\]

A one-way ANOVA (Analysis of Variance) was designed to test the hypothesis:

- Number of factors = 1
- Number of levels = 8 (“Al”, “25nm”, “50nm”, “75nm”, “100nm”, “150nm”, “200nm”, “250nm”, where the value in nm was the pore pitch.)
- Significance level = 5% ($\alpha = 0.05$)
- Normalising control, “G”, Glass, was used to adjust for varying cell culture conditions across runs.
5.3.2.1 Power Test

To estimate an appropriate sample size in the absence of existing results for porous alumina, data from other substrate types were used that had been tested using a similar cell culture protocol: For the required power, \( P = 1 - \beta > 0.99 \), a sample size of \( n \geq 4 \) gives the required \( \beta < 0.01 \).\(^{47}\) With \( n = 3 \) being the minimum number of runs to demonstrate reproducible results, it was decided to perform 4 runs, each with 2 substrates of one type, giving a total of \( n = 8 \).

For each substrate it was decided to evaluate the cell count from the mean of three images: one judged to be from an area with a typical number of cells, one with minimum number of cells and one with the maximum number of cells.

5.3.3 Results and Discussion

The data of Figure 47 show no significant difference between the aluminium and any one of the porous alumina substrates (Dunnet’s post-hoc tests, \( p \geq 0.282 \)) showing that the porous alumina films grown do not provide a surface for preferential NG108-15 cell vitality.\(^{48}\)

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\(^{47}\) where \( \beta \) is the probability of a Type II error (failing to reject the null hypothesis when it is false).

\(^{48}\) For comparison with other studies, it should be noted that the normalised glass control with a cell count of 100 is equivalent to 532 cells per mm\(^2\) (\( = 532 \times 10^6 \text{ m}^{-2}\)).
Figure 47. Cells counts on porous alumina ($n = 8$, 4 runs; each run was normalised to 100, based on the mean of 2 glass substrate cell counts; error bars are 95% confidence intervals ($\alpha = 0.05$))

It could not be determined at this stage why the 150 nm cell count was significantly lower than the 100, 200 and 250 nm substrates. It is possible that the cause relates to the porosity of the substrate, which was adjusted for the 150–250 nm substrates using a pore-widening etch.

As the first cell culture experiment, it was found that the procedure had several limitations:

1. Cell count method: the use of areas of minimum cell count frequently resulted in a cell count of zero; the selection of a maximum cell count often resulted in using an area with clumped cells that cause errors in the count. It was therefore decided that future experiments would use the means of $\geq 5$ images per substrate selected randomly from the substrate area. By increasing the number of images used, the error between their mean and the substrate mean could be reduced.

2. During the experiment it was found that cells were adhering to the underside of the glass controls, resulting in an over-count. An improvement was introduced for subsequent experiments whereby the underside of the glass was wiped clean using a cotton bud soaked with buffer solution whilst transferring the coverslips from stain to clean buffer.

3. Towards the end of the experiment it was found that some of the aluminium substrates had corroded: the corrosion always beginning at the cleaved edges. The signature was a corrosion path that appeared to follow a winding trail, possibly
relating to the aluminium crystal boundaries (Figure 48). The effect on cell count was unknown, but it was assumed at this stage that the corrosion products may have been toxic. Later experiments sealed the edges of the aluminium substrates.

It was considered whether the surface chemistry of the porous alumina films (Section 2.3) could have masked any effect of the morphology in the above experiment. This was the premise for the following set of experiments.

![Corrosion of aluminium](image)

**Figure 48. Corrosion of aluminium (Run 3, Al₁), initiating from edge of substrate. Scale bar is 1 mm.**

### 5.4 Experiments to Assess Surface Chemistry as Factors for Biocompatibility

#### 5.4.1 Introduction

To improve the sensitivity of the above experiment to the morphology of the substrates, it was hypothesised that improvements to the surface chemistry of the porous alumina might be required.
5.4.1.1 AlPO\textsubscript{4} Monolayer and HCl Etch

The formation of porous alumina using a phosphoric acid electrolyte (H\textsubscript{3}PO\textsubscript{4}) results in a monolayer of aluminium phosphate covering the surface [276],[277]:

\[
\text{Al}_2\text{O}_3 + 2\text{H}_3\text{PO}_4 \rightarrow 2\text{AlPO}_4 + 3\text{H}_2\text{O} \tag{12}
\]

The material presented to the cell in the previous experiment was therefore AlPO\textsubscript{4} rather than Al\textsubscript{2}O\textsubscript{3}. The AlPO\textsubscript{4} may itself be hydrated, presenting AlPO\textsubscript{4}·nH\textsubscript{2}O, where \( n \leq 1 \).

To test whether this layer had an effect on cell vitality and/or adhesion, it was proposed to remove this phosphate monolayer prior to cell culture. AlPO\textsubscript{4} may be selectively etched from Al\textsubscript{2}O\textsubscript{3} using hydrochloric acid [278],[279]. Since aluminium is soluble in HCl, this process relies on almost all the aluminium thin film being consumed during anodisation, otherwise the film might lift from the glass substrate. An etch of 32 wt% HCl, 20ºC, 60 s, was found to leave the film intact.

As an alternative to the HCl etch, the formation of the phosphate monolayer could be avoided by changing the electrolyte type. Anodisation using a 2 wt% oxalic acid was therefore investigated.

5.4.1.2 Trapped Charge and Annealing

It has been shown that anodisation of aluminium results in trapped charges within the alumina, with negative charges at the alumina surface and positive charges at the metal/oxide interface [280],[281]. Strong surface charges may cause excessively strong interaction with adhesion proteins resulting in denaturation\textsuperscript{49}. Since the charge density on the substrates produced was unknown, it was decided to test further substrates after neutralising the trapped charge. A 1 hour anneal at 200ºC has been shown by [282] to be sufficient to anneal these charges and are suitable conditions to avoid altering VLSI CMOS transistor characteristics or diminishing the reliability of any subsequent electrode design.

\textsuperscript{49} Denaturation is the loss of tertiary structure of a protein. The primary structure is a protein’s sequence of amino acids, the secondary structure is formed by the linking of hydrogen bonds along the length of a protein and the tertiary structure is the three-dimentional shape taken by a protein.
5.4.1.3 Anodic Barrier Films

To separate the morphology of the porous films from other factors such as trapped charge and hydration, it was decided to evaluate planar alumina substrates in the form of anodic barrier layers. These are, in effect, artificially thickened native oxides, with additional artefacts from the anodisation process. These were produced by galvanostatic anodisation in an electrolyte of 3 wt% ammonium tartrate at approximately 5–10 mA·cm\(^{-2}\), 22°C [218],[219]. By using the barrier anodisation ratio of 1.4 nm·V\(^{-1}\), different thicknesses of barrier layer could be produced by stopping the process once the required voltage had been reached.

5.4.1.4 Other Factors

Hydration of the substrate surface and the sterilisation protocol were also considered as factors that might have influenced cell vitality and adhesion. Hydration of alumina results in AlOOH and Al(OH)\(_3\) compounds that can dramatically change the morphology due to the formation of ‘whiskers’ or by ‘sealing’ at the pore entrances [276]. The level of hydration is affected by the post-anodising rinse procedure, storage humidity and cell culture conditions. Some hydration steps are reversible, and it is generally difficult to estimate the state of hydration during the experiment. There was also a question whether the sterilisation protocol could result in ethanol being retained in the pores of the alumina that could subsequently affect cell vitality and adhesion. However, the volume of any ethanol trapped was thought to be small in comparison to the 2 ml of growth medium applied, and therefore any residual ethanol concentration would probably be too low to cause a significant effect.

In order to evaluate further the above factors, supplementary experiments were first performed using small sample sizes. Potentially interesting substrates were then carried forward for a more extensive evaluation using a larger sample size. Two groups of substrates were to be evaluated: a set of porous morphologies and a set of planar barrier oxides.

5.4.2 Materials and Methods

As for the previous experiment, the null hypothesis was that there was no difference between number of cells (vitality) on native oxide and the alumina substrates. A one-way ANOVA was performed on the substrates of Table 7 to identify any potential effects for a further study using a larger sample size (number of factors = 1; \(\alpha = 0.05\); glass normalising control, “G”).
Table 7. Substrates tested having porous morphologies.

<table>
<thead>
<tr>
<th>Type</th>
<th>Pitch</th>
<th>Processing parameters</th>
<th>Identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td></td>
<td></td>
<td>G</td>
</tr>
<tr>
<td>Aluminium (control)</td>
<td></td>
<td></td>
<td>Al</td>
</tr>
<tr>
<td>Porous Alumina – Phosphoric</td>
<td>150 nm</td>
<td>not annealled</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>annealled</td>
<td>150A</td>
</tr>
<tr>
<td></td>
<td>200 nm</td>
<td>not annealled</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>annealled</td>
<td>200A</td>
</tr>
<tr>
<td>Porous Alumina - Oxalic</td>
<td>75 nm</td>
<td>not annealled</td>
<td>O75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>annealled</td>
<td>O75A</td>
</tr>
<tr>
<td></td>
<td>150 nm</td>
<td>not annealled</td>
<td>O150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>annealled</td>
<td>O150A</td>
</tr>
<tr>
<td></td>
<td>200 nm</td>
<td>not annealled</td>
<td>O200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>annealled</td>
<td>O200A</td>
</tr>
</tbody>
</table>

A further set of substrates was used to compare barrier layers (annealed and not annealed) with aluminium (Table 8).

Table 8. Barrier layers tested.

<table>
<thead>
<tr>
<th>Film Thickness</th>
<th>Processing parameters</th>
<th>Identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 nm</td>
<td>not annealled</td>
<td>B10</td>
</tr>
<tr>
<td></td>
<td>annealled</td>
<td>B10A</td>
</tr>
<tr>
<td>50 nm</td>
<td>not annealled</td>
<td>B50</td>
</tr>
<tr>
<td></td>
<td>annealled</td>
<td>B50A</td>
</tr>
<tr>
<td>100 nm</td>
<td>not annealled</td>
<td>B100</td>
</tr>
<tr>
<td></td>
<td>annealled</td>
<td>B100A</td>
</tr>
</tbody>
</table>

Opportunistically, two nanoporous titania substrates were also included as these were expected to present significantly different surface chemistries from all alumina samples: one was annealed at 400ºC and one at 500ºC resulting in different porous structures and oxide stoichiometries [283].

The follow-on tests with larger sample sizes were also 1-way ANOVA but with $n = 6$ over 3 runs: the null hypothesis was again that there was no difference between number of cells (vitality) on native oxide versus any one of the other alumina substrates. To enable direct

---

50 Titanium oxide
51 The two porous titania samples were supplied courtesy of Dr D. Regonini
52 Power of experiment estimated as per Section 5.3 ($\alpha=0.05$)
comparison with the initial biocompatibility test results of Section 5.3 above, the same pore pitches were evaluated, up to a maximum of 200 nm.\textsuperscript{53}

As a further refinement to the experimental method, the corrosion from the cleaved edges and its effects were eliminated by either coating the edges of aluminium substrates using a bio-inert silicone elastomer (‘732’, Dow Coming, UK) or by excluding corroded substrates from the results.

\textbf{5.4.3 Results}

The results presented in Figure 49 show significant differences between the aluminium and several of the porous alumina substrate types, most notably for annealled oxalic films (The gaps between the 95\% confidence interval bars and the aluminium control denote significant differences at $\alpha = 0.05$). Several of the barrier oxide substrates had a significantly higher cell count than the aluminium (Figure 50), although there were no significant differences between the annealed versus un-annealed types.

\textsuperscript{53} The 250 nm pitch was not included due to initial difficulties with fusing during room temperature anodisation.
Figure 49. Preliminary evaluation of annealing, electrolyte type and phosphate etch factors (normalised to glass cell count of 20; n = 2, 1 run): a. significant results (*) versus aluminium; b. grouped by processing; c. significance level, p, of differences between means (versus aluminium), where p < 0.05 is significant.
Figure 50. Preliminary evaluation of cell count on barrier layers (and porous titania) versus aluminium control (n = 2, 1 run).

Based upon the above results, porous alumina substrates produced using oxalic acid and annealed were selected for further testing. In addition, the barrier oxides, represented by B100A, were also taken forward.

However, in the follow-on tests using larger sample sizes, no significant difference was seen between the aluminium control and any of the anodised substrates (Figure 51). The reduced variance compared with the initial experiments of Section 5.3 is attributed to the improved cell count procedure (i.e. mean of 5 randomly-selected areas on a substrate rather than the “minimum-typical-maximum” selections using in Section 5.3). The increase in cell count on aluminium compared with previous experiments was attributed to the elimination of the corrosion effects by sealing the substrate edges with elastomer.

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54 The large variance of the 150 nm substrate was caused by a single clump of cells resulting in a single outlier.
Figure 5.1. Normalised cell counts on anodised substrates versus aluminium control (n = 6, 3 runs; normalised to a count of 10 on glass), showing no significant differences between means (p = 0.698).

5.4.4 Discussion

Although the refined procedure of this experiment as well as the results of previous experiments showed no significant difference in cell vitality for different substrate types, two other important observations were made:

Firstly, given the diversity of substrate morphologies and chemistries tested to date, it is unlikely that there is no effect on cell adhesion, even though these differences are not revealed by an evaluation based on cell count. However, since a tight cleft is based on good cell adhesion and is a prerequisite for good electrical coupling between cell and electrode, a more direct measurement of cell adhesion was sought. Since the importance of adhesion is central to the premise of this work, the pursuit of a suitable method formed the basis of the following set of experimental work (Chapter 6).

Secondly, the native oxide was shown to corrode in both the growth medium and buffer solutions, either during the experiment (at the cleaved edges) or afterwards (discolouration of the entire surface). This is an important consideration for the feasibility of CMOS electrode design since the corrosion products were shown to impact cell counts.
5.5 Corrosion Experiments

5.5.1 Introduction

The preceding experiments showed that at least three corrosion mechanisms act on the segments of aluminium coverslip: firstly, corrosion was seen initiating from the edge of the segment, resulting in a ‘winding’ corrosion path; secondly, a discoloration of the coverslips after 3–4 days in buffer solution; thirdly, white ’spotting’ was seen on coverslips after being used for cell culture.

Corrosion of CMOS electrodes has been briefly outlined in the literature but with no discussion of corrosion mechanisms [91],[160],[238],[284]. However, the understanding and resolution of these corrosion issues are essential if aluminium CMOS pads are to be used successfully for electrophysiology electrodes.

5.5.2 Methods and Results

5.5.2.1 Krebs Buffer

Two CMOS MEA ICs were incubated at approximately 40°C with culture chambers filled with Krebs buffer solution (NaCl 118 mM, NaHCO₃ 25 mM, KCl 4.8 mM, KH₂PO₄ 1.2 mM, MgSO₄ 1.2 mM, Glucose 11 mM, CaCl₂(2H₂O) 1.5 mM) in an air environment. Inspection after 48 hours showed several of the pads to have discoloured brown (Figure 52a).

5.5.2.2 Plating Medium

Two passive CMOS MEA ‘WET3’ ICs, each with 48 separate electrode pads, were plated with NG108-15 cells in growth medium and incubated at 37°C, 10% CO₂ for 24 hours. These MEAs had good die (i.e. electrodes were electrically connected to the package pins), but had been rejected after incorrect application of the culture chamber sealant. The medium was changed for plating medium and the cells incubated for a further 3 days. After this time, one of the MEAs had 8 pads that were visibly corroded (Figure 52b, c). All pads on the other MEA had corroded, except for one that appeared to be covered by a thin layer of sealant (Figure 52d, e).

55 From previous work by the University of Bath and King’s College London
5.5.2.3 Archived CMOS MEAs

On re-examination of previous work performed by The University of Bath and King’s College London, it was found that many pads had the ‘discoloration’ signature (Figure 52f). Additionally, the CMOS passive MEA used for bias experiments (Appendix A) had also corroded (Figure 52g).
Chapter 5 – Preliminary Materials and Biocompatibility Evaluations

Figure 52. Corrosion of CMOS metallisation.

a. CMOS MEA – after exposure to buffer: all pads corroded except for those arrowed

b. CMOS ‘WET3’ MEA – bias / Growth Medium (GM) before use (pad diameter = 30 Œm)

c. CMOS ‘WET3’ – bias/GM after use (discolouration marked)

d. CMOS ‘WET3’ before exposure to GM (pad diameter = 30 Œm)

e. CMOS WET3 after exposure to GM: all pads are discoloured except one (marked).

f. Archived unit from previous work by Bath/KCL – after tests (large pad diameter = 30 Œm).

g. Archived unit – after d.c. bias experiment. (pad diameter = 30 Œm)
5.5.3 Discussion

The white ‘spotting’ (Figure 52i) is distinctive of pitting corrosion. The spots propagate from localised defects on the surface, such as flaws in the native oxide and are frequently activated by aggressive ions such as chlorides. The sequence is initiated by dissolution of the aluminium into $\text{Al}^{3+}$ where it reacts with $\text{Cl}^-$ to form $\text{AlCl}_4^-$ [285]. As a result of $\text{H}^+$ formation, the base of the pit becomes acidic which enables further dissolution of aluminium. The $\text{Al}^{3+}$ ions diffuse out of the pit, where the less acidic environment enables precipitation in the form of aluminium hydroxide, $\text{Al(OH)}_3$. This white precipitate can be identified as the rings circling many of the pits in Figure 52i [285]. The overall corrosion reaction is described by (13):
The propagating edge corrosion (Figure 52k) is somewhat characteristic of intergranular corrosion, forming a network along grain boundaries [286]. This mechanism is typically enhanced by the presence of alloying elements that create second phase precipitates at grain boundaries. Although the aluminium is of high purity (99.9%), it is conceivable that an interaction with the underlying titanium barrier (e.g. alloying) layer could provide the required environment for corrosion. Whilst presently only seen propagating from edges, these observations do demonstrate that once this mechanism is initiated, the damage to an electrode is significant.

The cause of the discolouration of the aluminium surface, seen on both CMOS pads and aluminium coverslips, is as yet unknown. Due to its uniformity, it is possible that this is a thin film of corrosion product, but its potential to propagate into the remaining aluminium layer is undetermined. However, since the aluminium is particularly reactive in the presence of chloride ions, it is speculated that the corrosion may take the overall form of (14):

\[
\text{Al} + 3\text{Cl}^- \rightarrow \text{AlCl}_3 + 3\text{e}^-
\]

5.5.3.1 Solutions to Corrosion

A prerequisite to biocompatibility is having an electrochemically stable electrode (See Section 3.5). Various approaches to the problem of corrosion can be considered.

Firstly, cathodic protection can be used. But this would require a constant bias on the electrodes and so is likely to be problematic in an environment where an IC may be unpowered. The bias would also complicate the amplifier design and may cause undesirable effects with respect to cell adhesion and physiology.

Alternatively, coating with a noble metal, i.e. electroplating, can isolate the aluminium entirely from the solution, although if the interface is exposed to an electrolyte (e.g. through pinhole defects) it can lead to very rapid galvanic corrosion.
Lastly, an established method of corrosion prevention is, of course, anodisation. Indeed, for all of the experiments performed to date, no corrosion of any of the anodised sample has been recorded.

5.5.3.1.1 Anodisation

The experiments have shown that the growth of a planar barrier layer protects the aluminium, but the highly insulating oxide would require modification to become conductive in order to make a useful electrode. Two methods that are compatible with the low-cost post-processing constraints are the introduction of leakage paths by increasing the defect density of the oxide, and ion implantation. The study of defect density in alumina is fairly mature and increasing the defect density may be possible by simple modification of the anodisation electrolyte \[^{282,287}\]. Ion implantation of layers up to 1 µm can be achieved, but has the disadvantages of requiring costly equipment, requires a high temperature drive-in to activate the defects, and will implant the IC passivation as well as the pads and therefore may cause increased electrical leakage.

Alternatively, a *porous* alumina layer can be used to provide the same corrosion inhibition as a planar barrier oxide. However, an advantage of the porous layer is that there is only a thin barrier oxide at the base of each pore. This is more easily modified to produce a conductive interface. As previously discussed in Section 3.2.2.1, the barrier can be thinned by a post-anodisation etch, can be thinned by stepping down the voltage towards the end of anodisation, or by coating with a noble metal that tends to alloy with the oxide forming a conductive path to the underlying metal.

5.5.3.1.2 Electroplating

As reviewed in Section 3.2.3, plating with gold has already been achieved by others to form low-cost CMOS electrodes, but only at the expense of introducing the hazard of rapid galvanic corrosion at the gold-aluminium interface \[^{238,242}\]. Shifted electrodes using platinum have also been successfully developed but the IC processing, including photolithography steps, means this approach does not meet the low-cost requirement. However, as envisaged in Section 3.1, it is possible that a noble metal could be used to coat a
porous alumina electrode, having the corrosion prevention of the alumina combined with the electrochemical stability of the noble metal surface.

5.6 Conclusions

CMOS metallisation has been reproduced on glass and successfully anodised. The thin film anodisation process has been characterised electrically. Scaling factors for CMOS anodisation have been calculated and indicate on-chip anodisation should be possible without causing electrical overstress. Anodising of a true CMOS IC pad was demonstrated.

The biocompatibility of porous alumina has been demonstrated using the NG108-15 cell line. Experiments evaluating cell vitality showed no significant difference between aluminium controls and the porous alumina substrates.

These experiments did not show any difference between the various porous alumina pore pitches or various surface chemistries, yet it was postulated that a difference in adhesion across such a range of morphologies is more likely to exist than not. It was therefore surmised that an improved experimental method was required. Since the importance of adhesion is central to the premise of this work, the pursuit of a suitable method formed the basis of the following experiments (Chapter 6).

Corrosion has been identified as a significant limitation of unmodified CMOS pads, leading to deterioration of the electrode and harmful corrosion products. Prevention of corrosion has therefore been established as a key requirement of a successful CMOS electrode. Porous alumina was tested in physiological media and showed no evidence of corrosion. As a novel approach to electrode design, it should be possible to combine the benefits of anodisation with those of electroplating: firstly, the consumption of the aluminium layer by anodisation removes the corrosion source; secondly, by selecting a porous layer, all the aluminium can be anodised, leaving only a thin or remnant barrier layer; and thirdly, the coating of the alumina with a noble metal will protect any remaining islands of aluminium that have not been anodised, may alloy with any remaining barrier oxide to form a conductive path, and may be readily coated with platinum black to present a low-impedance electrode.
Key Points

- Anodisation of CMOS pads has been demonstrated for the first time. Modelling indicates that on-chip anodisation is possible without causing electrical overstress.
- Porous alumina has been demonstrated to be biocompatible with a neuronal cell line.
- Prevention of corrosion is a key consideration in the modification of CMOS electrodes for use in cell-based biosensors.
- New experimental methods are required to study the effect of substrate morphology on cell adhesion.
6 Cell Adhesion

6.1 Adhesion Measurement

It was shown in the preceding chapter that neuronal cell counts do not correlate with cell-substrate adhesion. This is supported by [288] where cell counts and adhesion also showed no correlation. Adhesion can be measured either by direct shear measurement [289] or indirectly via laminar flow shear stress.

Cell-substrate shear stress has often been measured in three ways: firstly, a spinning disk can be used to relate linear radial increase of fluid shear stress to cell detachment [290]. Secondly, a commercial multi-well plate centrifuge can be used for a detachment assay [291]. Lastly, a flow chamber can be used to relate fluid velocity to shear stress [292]–[294]. The simplest method to apply to the cell culture protocol used here, and the most developed, is the flow chamber. It was therefore decided to study the flow chamber design in more detail, with the intention of developing a cost-effective solution to measuring NG108-15 cell adhesion.

6.2 Parallel Plate Flow Chamber

A flow chamber design has already been developed specifically for the measurement of NG108-15 cells [288]. Expected shear stress magnitudes are therefore already understood for this cell line. However, the flow chamber in that study was of a variable height design – a feature subsequently unused – and a refinement not required for this work.

A useful feature of many flow chamber designs is the incorporation of a gasket to define the chamber height. This allows the height to be varied by simply changing to a different thickness of gasket [295]–[297]. Other designs use O-ring seals that enable fast vacuum clamping of a lid, but the chamber height is then fixed [298].

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56 With other variables held constant, a change of chamber height results in a change of flow rate. Changing the gasket therefore represents a simple method of adjusting the range of flow rates without the need to re-machine the chamber body.
6.2.1 Methods

The design developed for this work (Figure 53) was loosely based on [288] and [295]. The chamber features a well in the base, of a thickness that matches the height of the coverslip substrates. This provides a uniform base without discontinuities and so ensures the laminar flow is not broken as the fluid passes onto the substrate. For efficient use of the anodised coverslips and to allow continued use of the submersed electrode anodisation technique, the design allows part-coverslips to be used, but the design does require at least one straight side to the segment to butt against the wall of the well on the upstream side of the chamber. The chamber is formed from a cut-out in the gasket, the width being defined by the size of the coverslip segment. Most importantly, the design allows real-time microscopy of the slide using the epi-illumination microscope.

A comparative study of flow chamber design has shown that inlets that are inline with the channel are most efficient at establishing laminar flow near the end of the chamber [299]. This is important to ensure flow over the substrate under test is laminar and to keep chambers as short as possible as so avoid unnecessarily high fluid pressures. Such inlets were therefore incorporated into the design used here, illustrated by Figure 53 and Figure 54.

![Figure 53. Design schematic for the parallel plate flow chamber.](image)
In order to estimate an appropriate chamber height, it is necessary to model the fluid flow, as described by [299]. A key parameter is the Reynolds Number, $Re$, for which values up to approximately 1400 represent laminar flow.

The Reynolds Number, $Re$, is defined by (15), where $\rho$ is the fluid density, $Q_{pp}$ is the flow rate, $w_0$ is the chamber width, $h_0$ is the chamber height and $\eta$ is the absolute viscosity.

$$Re = \frac{\rho Q_{pp}}{(w_0 + h_0)\eta} \quad (15)$$

Since the maximum shear stress required to displace adhered NG108-15 cells has been determined in [288], this can be used to determine the flow rate using the relationship given in (16) and (17), where $\sigma$ is the shear rate and $\tau_w$ is the shear stress. The choice of peak flow rate is influenced by the need to be efficient in the use of buffer solution and by the specification of the pump.

$$\sigma = \frac{3Q_{pp}}{2(\frac{h_0}{2})^2w_0} \quad (16)$$

$$\tau_w = \eta \sigma \quad (17)$$
With $\tau_w = 14.0 \text{ Nm}^{-2}$ (140 dynes cm$^{-2}$), $\rho = 9.98 \times 10^2 \text{ kg m}^{-3}$, $\eta = 1.00 \times 10^{-3} \text{ kg m}^{-1} \text{s}^{-1}$ (assuming the experiment is to be performed at room temperature), $w_0 = 7 \text{ mm}$, it is possible to determine values for $Q_{pp}$ and $Re$ for varying chamber heights $h_0$. To allow fast modelling of various heights and flow rates, the above model was recreated as spreadsheet formulae (Microsoft Excel 2003). A height of 0.5 mm should give a suitably low flow rate of 56 ml min$^{-1}$ whilst maintaining laminar flow with $Re = 776$. A height of 0.2 mm should result in 350 ml min$^{-1}$ with $Re = 129$. These height calculations allowed thin sheet materials to be used as gaskets. The channels were cut with a modelling knife and steel rule guide, thereby ensuring the sides were straight – this being important to ensure the flow remains laminar.

For calibration of the equipment, NG108-15 cells were cultured on plain glass coverslips for 3 days as per the protocol outlined in Section 5.3.1 and then stained according to Section 5.3.1.2 immediately before testing. Coverslips were loaded into the chamber and perfused with recording buffer solution (Section 5.3.1.2). The flow of buffer was provided by a peristaltic pump (520S, Watson Marlow, Wilmington, U.S.). Flow times were initially defined as 5 s, as used in [288]. Experiments were carried out at room temperature.

### 6.2.2 Results and Discussion

The flow chamber failed to detach a significant proportion of the NG108-15 cells from the glass coverslips. Cells remained attached despite the flow being increased far beyond the maximum rate expected to maintain laminar flow. Increasing the duration of the stress beyond 5 s (up to 20 mins) also failed to detach cells. Instead, the stain was noted to leach from the cells under flow (Figure 55).
At first examination, it would be expected that the above flow chamber would give comparable results to those in [288]. However, closer inspection of [287] shows that cells underwent detachment testing when the cells were already losing vitality and so were more readily detached from the substrates. Alternatively, it is conceivable that the adhesion of cells to glass is considerably greater than to all the five substrate types used in [287].

It was therefore concluded there are two limitations of the parallel plate flow chamber that prevent its use under the conditions of interest here. Firstly, we are interested in measuring the long-term adhesion of the cells on a working electrode (several days to weeks) rather than the short-term adhesion processes (hours) that are usually evaluated using a flow chamber. Adhesion strengths greatly increase over several days in-vitro [300] and flow chambers can then no longer provide sufficient laminar flow to cause detachment. Shear strength may again
decrease to measurable levels for cells plated for extended durations where they have lost vitality (as in [287]), but this is of little interest in the context of electrophysiology. Secondly, under high flow, stain leached from the cells causing difficulty in detecting those cells that remained adhered.

Due to the above drawbacks, alternative cell adhesion assays were considered. Other methods for quantifying adhesion [301] include the rotating disk [290],[302], jet impingement [303]–[306] and centrifugation [307]. Jet impingement was assessed using syringe needles (G19 gauge), syringe pump and cells on glass coverslips, but trials suggested that obtaining reproducible results would be difficult. The rotating disk would require fabrication of specialist equipment and would require substrates larger than available with coverslips: a rotating disk assay was therefore not pursued. Centrifugation was chosen for further investigation due to its potential simplicity and access to appropriate equipment.

6.3 Centrifugation

6.3.1 Introduction

Adhesion of a cell to a substrate is mediated by its protein receptors. These form bonds with forces normal to the surface. As discussed above, several methods have been developed to assess this adhesive force. However, nearly all of the methods quantify adhesion by applying a shear force rather than a force applied normally to the substrate (See Section 6.1 above). This is probably for two reasons: firstly, the flow of fluids across adhesive cells is a natural in-vivo process such as with blood cells and across the walls of capillaries; secondly, it is difficult to configure an assay where the force is away from the substrate but much easier to apply shear forces using a flow of fluid (e.g. the parallel plate flow chamber) or centrifugation (e.g. spinning disk). In fact centrifugation does have the potential to apply a normal detachment force but this requires the substrate to be mounted at it edges or back side, with the cells suspended outwards (i.e. facing away from the spinning axis) [308]. Regardless of the substrate orientation, a detachment force must overcome the normally-orientated cell-substrate adhesion force, as illustrated in Figure 56. It can be seen that the torque of the cell produces a peeling action from the edges of the adhered membrane. This is a similar action to that of a parallel plate flow chamber or cantilever assays [309],[310]. Further inspection of a
centrifugation assay using a normal detachment force shows that detachment in this configuration is also a peeling force [308].

Figure 56. A simple ‘point model’ for centrifugation of a cell. The cell is shown attached and in equilibrium with its substrate. Adhesive forces, $\sigma$, balance the normal reaction, $s$, of the substrate and the torque resulting from the centrifugal force. The centrifugal force, $F_c$, is balanced by the reaction, $F_x$, which represents a torque around the centre of the cell, $r_c$, which must also be balanced by adhesive forces, $\sigma$. (Adapted from [311]).

Due to the strong cell-substrate adhesion observed in the preceding flow chamber experiments, a centrifugation assay was designed to enable larger shear stresses than other centrifugation assays [312],[313], with substrates mounted in the same plane as centrifuge rotation. The acceleration, $a$, on a cell is then given by:

$$a = \omega^2 (r + x)$$  \hspace{1cm} (18)

where $r$ is the radius from centrifuge axis to inner edge of the substrate, $x$ is the distance of a cell from the inner edge of the substrate, and $\omega$ is the rotational speed. With small substrates of approximately 1 cm length, $r >> x$ and so the relationship can be simplified to $a = \omega^2 r$. With centrifuge speeds expressed in revolutions per minute (rpm), and with $\omega = 2\pi(rpm) / 60$, the acceleration can be stated more usefully as:

$$a = \left(\frac{\pi (rpm)}{30}\right)^2 r$$ \hspace{1cm} (19)

This acceleration is often expressed as a relative centrifugal force (RCF), $RCF = a/g$, where $g = 9.81 \text{ m/s}^2$. If required, the shear force on a cell, $F_s$, can then be determined by the relationship:
\[ F_s = v_{cell}(\rho_{cell} - \rho_{medium})g \]  

(20)

where \(v_{cell}\) is the volume of a cell, \(\rho_{cell}\) the density of a cell, and \(\rho_{medium}\) the density of the medium. With a physiological medium of density \(\rho_{medium} = 1.00 \times 10^3 \text{ kg m}^{-3}\) and an estimated cell density \(\rho_{cell} = 1.07 \times 10^3 \text{ kg m}^{-3}\) [313], it can be seen that the cell’s effective weight in medium (i.e. the detachment force, \(F_s\)) is small compared with its weight in air, \(v_{cell} \rho_{cell} g\). Centrifugation assays have been performed in air by [312] to increase the detachment force, but are undesirable here as the cells would no longer be under physiological conditions.

### 6.3.2 Materials and Methods

Porous alumina morphologies chosen for centrifugation (17 nm, 69 nm and 206 nm) were guided by those used in Sections 5.3 and 5.4. The set was limited due to the time required for the repetitive centrifugations. The Keithley 236 Source-Measure Unit provided the anodisation potential. For potentials up to and including 60 V the anodisation was performed at 25°C in a 4 wt% phosphoric acid electrolyte. For potentials greater than 60 V, anodisation was performed at 10°C with the electrolyte diluted with 25% v/v ethanol to avoid localised burning and metal fusing. Pore widening was performed using 4 wt% phosphoric acid at 45°C. A scanning electron microscope (Hitachi S-4300) was used to check the surfaces produced and the pore pitches were estimated from the images by the method discussed in Section 5.2.3.3. Additionally, any monolayer of aluminium phosphate (AlPO₄) covering the surface was selectively etched from the alumina using hydrochloric acid (32 wt% HCl, 20°C, 60 s), as discussed in 5.4.1.1 and surface charges annealed by baking for one hour at 200°C as discussed in Section 5.4.1.2. Cells were cultured according to the protocol of Section 5.3.

The coverslip substrates were cleaved into approximately six squares of area ~1.0 cm² using a diamond scribe. Corrosion at the cleaved faces of aluminium substrates was prevented by coating the edges with either a bioinert silicone sealant (732, Dow Corning) or varnish (‘TRV’, Electrolube, UK). Sterilisation of the substrates was performed in the laminar flow hood by submerging in ethanol for 30 minutes followed by air drying for 15 minutes. The substrates were subsequently moved to sterile 35 mm dishes onto which 2 ml of cells (at a density of 10,000–20,000 ml⁻¹) were plated out and incubated at 37°C, 10% CO₂. Growth
medium was replaced after 24 hours with plating medium and the cells incubated for a further three days prior to centrifugation tests.

Due to the opacity of the aluminium substrates, it was necessary to use the epi-illumination microscope and use the methylene blue cell staining protocol discussed in 5.3.1.2.

In order to create sufficient detachment forces, it was necessary to use high centrifuge speeds. A bucket rotor provides orthogonal rotation of tubes and therefore gives simplicity of design but buckets are limited to relatively low rotation speeds. It was therefore necessary to design the centrifugation assay using a fixed angle rotor (Sigma-Aldrich 3K-30 centrifuge; 6 x 50 ml angle rotor). Holders were designed to present the substrates in an almost horizontal position within the angled centrifuge rotor so that the centrifugal acceleration was primarily in line with the plane of the substrate and so creating a shear detachment force (Figure 57).

The substrates were angled very slightly (<3°) to prevent them from flipping up onto the sidewall of the holder during centrifugation. This angle reduced the shear force by only ~0.1% (i.e. 1 − cos 3°) and so an acceptable approximation was to consider the coverslips as horizontal.
The holder design was found to be adequate up to speeds of 18,000 rpm. Breakages occurred above this speed due to the entire weight of the substrate being transferred via its two outside corners. If required, higher speeds could be obtainable by designing a holder to distribute the weight along the entire outer edge of the glass.

Preliminary experiments suffered the same methylene blue stain loss problem as encountered during the flow chamber experiments (Section 6.2.2). The problem was resolved simply by centrifugation in buffer containing methylene blue (5% w/v) instead of plain buffer. Centrifugation was performed at 20°C with the set speed maintained for 5 minutes. Ramping of centrifuge speed was controlled to avoid disturbance of the substrates.

### 6.3.3 Results and Discussion

The porous alumina substrates to be used in the experiment were evaluated by analysis of scanning electron microscope images using ImageJ software. The substrate characteristics are summarised in Table 9 along with data published in the literature for comparison.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Anodising Voltage (V)</th>
<th>Mean Pore Pitch (nm)</th>
<th>Porosity prior to pore-widening (%)</th>
<th>Final Porosity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘17 nm’</td>
<td>10</td>
<td>17</td>
<td>63*</td>
<td>63</td>
</tr>
<tr>
<td>‘69 nm’</td>
<td>40</td>
<td>69</td>
<td>22</td>
<td>59</td>
</tr>
<tr>
<td>‘206 nm’</td>
<td>120</td>
<td>206</td>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td>Hoess et al. [207] - 40 V (oxalic electrolyte)</td>
<td></td>
<td></td>
<td></td>
<td>48</td>
</tr>
<tr>
<td>Hoess et al. [207] - 150 V (phosphoric electrolyte)</td>
<td></td>
<td></td>
<td></td>
<td>39</td>
</tr>
<tr>
<td>Karlsson et al. [205]</td>
<td></td>
<td></td>
<td></td>
<td>45</td>
</tr>
<tr>
<td>Karlsson et al. [208] - 20 nm pore diameter</td>
<td></td>
<td></td>
<td></td>
<td>45</td>
</tr>
<tr>
<td>Karlsson et al. [208] - 200 nm pore diameter</td>
<td></td>
<td></td>
<td></td>
<td>47</td>
</tr>
<tr>
<td>Sapelkin et al. [181] - pSi</td>
<td></td>
<td></td>
<td></td>
<td>28</td>
</tr>
</tbody>
</table>

Table 9. Characteristics of alumina substrates used for centrifugation and comparison with other studies (* denotes pores not widened).
Detachment of cells was treated using statistical methods for survival [314]. Data were fitted to a 3-parameter Weibull distribution, with a threshold of zero (i.e. no failing cells at 0 rpm since unadhered cells were removed during handling); the same Weibull shape parameter was applied to all substrates whilst maintaining an acceptable goodness of fit ($R^2 \geq 0.887$). Figure 58 shows these data as a percentage of cells detached as a function of centrifugation speed. The solid symbols represent experimental data while the lines represent the data fitted to the Weibull distributions. Each fit is shown as a set of three curves representing mean, upper confidence interval and lower confidence interval.

![Graph showing cell detachment as a function of centrifugation speed.](image)

Figure 58. Cell detachment profiles for 17 nm, 69 nm and 206 nm porous alumina and aluminium substrates ($n = 8$, 4 runs). Data were arbitrarily censored. Curves are means and 95% confidence intervals for fits to Weibull distributions (threshold = 0, shape = 0.9381).

A comparison of the above curves was performed by ANOVA of the 50% cell detachment points, $\tau_{50}$, as shown in Table 10 and Figure 59. The 17 nm and 69 nm alumina substrates showed significantly poorer adhesion than the aluminium, whereas cells adhered significantly better to the 206 nm alumina compared to aluminium. The relative centrifugal force (RCF) can be calculated using Eqn (19) and is shown as the second y axis in Figure 59.
### Table 10

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$\tau_{50}$ / rpm</th>
<th>Standard Error (SE) at $\tau_{50}$ / rpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>8779</td>
<td>231.8</td>
</tr>
<tr>
<td>17 nm</td>
<td>719</td>
<td>51.1</td>
</tr>
<tr>
<td>69 nm</td>
<td>676</td>
<td>35.2</td>
</tr>
<tr>
<td>206 nm</td>
<td>15662</td>
<td>487.2</td>
</tr>
</tbody>
</table>

Table 10. $\tau_{50}$ and standard errors for porous alumina substrates and aluminium control.

### Figure 59

![Figure 59](image_url)

Figure 59. Analysis of $\tau_{50}$ for aluminium control and porous alumina substrates ($n = 8$, 4 runs) showing significant (*) differences ($p < 0.05$). Error bars represent 95% confidence intervals.

### 6.3.4 Discussion

The centrifugation adhesion assay demonstrated that pore pitch modulates the ability for neuronal cells to adhere to the surface. This corroborates [288] in finding that vitality is not a good indicator of adhesion.

The above results are also in broad agreement with the long-term adhesion study of [312] where Vero Green Monkey kidney (fibroblast-like) cells had a mean long-term detachment force that approached a maximum RCF of approximately 5000 after 25 hours. However, the majority of other quantitative adhesion tests have assessed only short-term processes which are of little relevance in the context of biosensor electrodes.
An additional problem arises in comparing centrifugation results with other methods due to the difficulty in evaluating absolute detachment force: for example, the short-term (12 hour) adhesion of NG108-15 cells on untreated glass was measured in [290] to have a mean detachment shear of 0.67 Nm$^{-2}$ (6.7±0.23 dyn·cm$^{-2}$). From these data, the absolute detachment force on a single cell can be determined only if the surface area of the cell exposed to the moving fluid is known. This was attempted in [315] by using assumptions of cell morphology (that cells were spherical) and an estimate of 10% for the proportion of cell surface in contact with the substrate. However, this figure has latterly been shown to be too low, with the proportion more likely to range between 12–40% (mean of 32%) for HEK293 cells on uncoated glass [316] and so demonstrates the difficulty in using such an estimate to quantify force. Similarly for centrifugation, calculation of detachment force via the aforementioned relationship, using Eqn (20), would require the cell volume, $v_{cell}$, to be determined.

Comparison between fluid shear and centrifugation methods could be made via the relationship between cell volume and cell surface area, but this requires quantitative evaluation of cell morphology (i.e. degree of cell flattening) and cell contact area. Because of these difficulties, a quantitative comparison between shear and RCF data is likely to result in unacceptable inaccuracy and so has not been attempted.

### 6.3.4.1 Pore Pitch

To explain the difference in adhesion of the smaller pore pitches (17 nm, 69 nm) compared to the larger pore pitch (206 nm), the porosity and morphology were considered.

The porosity of a substrate surface is the ratio of pore area to surface area. Without pore-widening, substrates with smaller pore pitches (e.g. 17 nm and 69 nm) have higher porosities than substrates with large pore pitches. Below a cell body, these highly porous substrates will present mainly physiological medium (i.e. within the pores) and these areas can provide no immediate adhesion: cell adhesion molecules can bond only with the surface formed by the tops of the thin pore walls. Secondly, it is conceivable that the morphology of the alumina could modulate adhesion, as noted in [205] where cell processes were observed to enter the larger pores of 200 nm diameter, but not into pores of less than 100 nm – it was perceived that the larger pores may provide anchorage points. Additionally, the native aluminium oxide
clearly cannot present such anchorage yet it has been shown to provide good adhesion. However, it might not be appropriate to make deductions regarding adhesion mechanism by direct comparison of the porous alumina and native oxide surfaces: for example, the stoichiometries of the artificial and native oxides are likely to differ; it is also conceivable that surface charge may be different even though this charge was believed to be removed by the annealling step. Alternatively, one could adopt a view that considers the planar native oxide surface as a porous alumina with zero porosity and zero pore diameter. In this case it would be valid to make deductions by comparing adhesion to native oxide and porous alumina substrates. In this scenario it can be postulated that for this combination of neuronal cells and porous alumina, it is the proportion of porous alumina surface (porosity) that modulates adhesion rather than surface morphology. This question forms the basis for the physical analysis of Section 6.4.

6.3.5 Conclusion
A novel centrifugation assay was developed to measure long-term cell adhesion strength of various porous alumina pore pitches versus a plain aluminium surface. Small pore pitches of 17 nm and 69 nm result in poor cell adhesion whereas a large pore pitch of 206 nm presents good adhesion with slightly better performance than the planar aluminium surface of an unmodified CMOS electrode.

6.4 Physical Analysis of Cleft

6.4.1 Introduction
As discussed above in Section 6.3.4.1, the differences in cell-substrate adhesion observed in the centrifugation tests could be due to either:

i. the porosity of the porous alumina (i.e. the proportion of surface presented by each substrate), or,

ii. the morphology of the porous alumina

In [205] cell processes were observed to enter the larger pores of 200 nm diameter but not into pores of less than 100 nm diameter (Figure 60). It was believed that the larger pores may provide anchorage points.
Figure 60. The interaction of human osteoblast-like (HOB) cells with nano-porous alumina: a. SEM showing the ends of cell protrusions (filopodia) entering 200 nm wide pores (scale bar is 2 µm); b. TEM of a single filopodium (marked) entering a pore (~200 nm wide, dark area). The cell body lies across the top of the image (Magnification not stated). From [205].

In [317] it was shown using TEM sections that the cleft is modulated by the type of adhesion protein used to coat a substrate. HEK293 (neuronal) cells were cultured on silicon substrates where the minimum measured cleft was found to be 35–40 nm when coated with poly-l-lysine. When using other proteins (e.g., fibronectin and laminin) or uncoated, the cleft was extremely irregular, preventing the calculation of a mean cleft size (Figure 61).

Figure 61. TEM images of HEK293 cells on silicon substrates: a. a silicon and gold substrate not coated with any adhesion proteins. The gold is the black line on the silicon – the black line is not the cleft. The cleft (arrowed) is the irregular lighter areas above the black line. The dark cell membrane is just discernable; b. interface coated with poly-l-lysine showing a more regular cleft of approximately 35–40 nm (arrowed). (Scale bars are 250 nm). From [317].

As a preliminary investigation of the adhesion of NG108-15 neuronal cells to porous alumina, substrates used in the preceding experiments were used for TEM analysis. The object was to image the cleft to determine if there was any signature that related to either of the proposed
causes of enhanced adhesion on the substrates with larger (206 nm) pore pitches and the poor adhesion observed with the small pores (69 nm).

### 6.4.2 Methods

NG108-15 cells were cultured and plated onto aluminium (control) and porous alumina substrates with small (69 nm) and large (172 nm) pore pitches, produced by anodising at 40 V and 100 V, respectively. The cell culture and substrate preparation protocols were the same as used previously (Section 5.3.1). Cells were prepared by rinsing in recording buffer and fixed using 2% glutaraldehyde in recording buffer. After incubating at 4°C for 2 hours, the substrates were rinsed in de-ionised water and the cells dehydrated using an ethanol series (70%, 85%, 95% and 100% ethanol) with a dwell time of ~1 min for each step. Substrates were sputter coated with gold or platinum for SEM imaging. Cells with a flattened, spreading morphology were selected for sectioning. The FIB of the dual-beam Carl Zeiss 1540XB system was used to section the cell (Figure 62). Ion beam deposition was used to coat a thick layer onto the top of the cell to improve strength of the sample and a location for attaching a microprobe. A further FIB cut produced a thick section (lamella) to which a microprobe was attached using the ion beam deposition capabilities of the 1540XB. The microprobe was to act as a handle. Further FIB cuts released the lamella. The lamella was then transferred to a TEM mount and sufficiently thinned using the FIB to allow electron tunnelling. The samples were then transferred to the TEM for imaging.
Figure 62. TEM lamella preparation: a. SEM top-view of cells on an aluminium substrate. The centre shows two FIB cuts with a thick section of cell across the centre. The remaining part of the sectioned cell can be seen to the right of the cut (Scale bar is 10 µm); b. the same section after attaching the microprobe. The lamella is in the process of being lifted away from the substrate (Scale bar is 10 µm); c. SEM image showing the lamella transferred to a TEM mount. The dark area at the base of the sample is the glass substrate with the porous layer clearly visible in the centre, above which is the cell (Scale bar is ~2 µm).

### 6.4.3 Results and Discussion

The TEM images are shown in Figure 63. The interface with the aluminium substrate shows no cleft (as discernable at the ~2 nm resolution of the TEM). For the alumina with large pores, the precise location of the interface is not particularly clear. However, protrusions from the cell are visible, and these align to the openings at the top of each pore. This was confirmed in several other images of this lamella. The TEM of the interface with ‘small’ pores shows the membrane to arch upwards over the pore openings.
The results represent two significant findings:

i. The lack of cleft on the aluminium substrate is unexpected and is in disagreement with the work of [317]. It is also in disagreement with other literature which confirms the presence of a cleft on silicon substrates (e.g. [64], Section 2.5). The lack of cleft on the aluminium substrate, if correct, could explain the good adhesion displayed in the centrifugation experiments. This needs confirmation through further work.

ii. The protrusions into the substrate with ‘large’ pores could be indicative of an anchoring mechanism that would explain the enhanced adhesion to these substrates. This would corroborate the anchoring mechanism proposed in [205] where osteoblast filopodia entered large pores. This contrasts with the ‘small’ pores where there are no
protrusions into the pores and, instead, the membrane arches upwards away from the substrate. These observations are new, but need confirmation through further work.

Further scrutiny of these preliminary results must question their validity. Firstly, it was noted that the internal cell structures (e.g. nucleus, mitochondria) were not visible. This might be explained simply by the lack of post-fix staining, often performed using OTOTO (OsO$_4$ / thiocarbohydrazide / OsO$_4$ / thiocarbohydrazide / OsO$_4$) [318],[319] or just OsO4 [317]. Alternatively, the lack of visible cell structures could be indicative of damage to the cell during the specimen preparation. Secondly, it is possible that the interfaces (clefts) of the specimens were distorted during the preparation sequence, either by the dehydration protocol or by the FIB sectioning. The protocol used for preparation had previously been used successfully at Cardiff University for the sectioning and TEM imaging of blood cells. However, it is noted that the specimens used in [317] to image the cleft were embedded in epoxy resin prior to sectioning. However, the only role of the epoxy in that work was to allow cleaving of sections and not stabilise cell structures. (The epoxy was not required for this role in our work as the dual beam SEM/FIB enabled precision sectioning and lamella extraction.) In [320], TEM specimens prepared using FIB sectioning were also embedded in resin. But successful TEM specimens have also been prepared by FIB without resin [319]. These doubts need addressing through further consideration or analysis before the above results are published.

In summary, this physical analysis suggested there is no cleft between aluminium and an NG108-15 neuronal cell. Secondly, the interface to alumina with large pores with a 172 nm pitch showed protrusions entering the pores that could explain the enhanced adhesion. This contrasts with cells on alumina with small pores (69 nm pitch) where no protrusions were observed and the membrane arches away from the substrate above each pore. To test whether the interface had been distorted, it is proposed that further work should confirm the suitability of the cell preparation protocol. This could include controls using protein coatings (e.g. laminin) that are known to cause a significant cleft [317].
6.5 Conclusions

Good adhesion of neuronal cells to an electrode is a prerequisite for successful extracellular recordings. In Chapter 5 it was shown that cell counts are an indication of vitality but do not correspond to the adhesive force of cells to a surface. A parallel plate flow chamber was assessed as an established method that is a more direct measurement of cell-substrate adhesion. This demonstrated that flow chambers are only useful for measuring low adhesion forces such as found when cells have been plated onto substrates for only a few hours.

A novel centrifugation assay was developed to measure the adhesion of cells that had been plated onto substrates for 3 days. This demonstrated that cells adhere better to porous alumina having a pore pitch of 206 nm than to aluminium controls. Cells adhered less well to porous alumina with small pore pitches of 17 nm and 69 nm. It was hypothesised that the observed adhesion characteristics might be either due to the porosity of the substrates or due to the size of the pores that might enable an anchoring mechanism. To investigate these hypotheses, TEM specimens were prepared using a dual beam SEM/FIB. Preliminary results indicated the cell protrudes into the large pores (206 nm pitch) but not small pores (69 nm pitch). Additionally, TEM of a cell on an aluminium substrate indicated there was no discernable cleft: this result is in disagreement with the literature. Both results warrant further investigation.

New methods have been developed that aid the assessment of cell adhesion to porous and planar substrates.

Having established the biocompatibility and adhesion characteristics of porous alumina, attention was turned to the electrical design of the electrode.
Key Points

- Cell counts measure vitality but not cell-substrate adhesion.
- The parallel plate flow chamber has been demonstrated as being inadequate for measuring long-term (3 day) cell-substrate adhesion.
- A novel cell-substrate adhesion assay has been developed that is capable of measuring long-term adhesion.
- Cells adhere preferentially to porous alumina with a large pore pitch versus aluminium and small pore pitches.
- TEM analysis indicates protrusions from the cell on porous alumina with large pore pitches could represent an anchoring mechanism that is responsible for the enhanced adhesion.
7 IC Design and Assembly

7.1 Introduction

Having addressed the principal biological aspects of porous alumina, consideration was given to the electrical design. A prototype CMOS MEA was to be manufactured using the semiconductor fabrication facilities accessible to educational establishments via the EUROPRACTICE initiative [321]. This European Commission programme enables universities to share a silicon wafer so that the mask production and manufacturing costs are also shared. New projects can only be submitted a few times each year, strict deadlines apply and lead-time is approximately three months. It was therefore necessary to submit the IC design at an early stage of the research.

7.1.1 IC Design

The CMOS pad post-processing procedures had not been developed at this stage. However, the model for the scaling of anodising currents to CMOS geometries had been developed (Section 5.2.3) and so there was sufficient confidence in the IC requirements to submit a design.

7.1.2 Prototype Packaging

EUROPRACTICE had previously been used by the University of Bath to fabricate the ‘WET3’ and earlier CMOS MEA prototypes. These devices had been supplied by EUROPRACTICE in open-cavity ceramic DIP packages. Assembly had been completed at the University of Bath and King’s College London by covering the bondwires with elastomer which was applied by hand. Due to the small geometries of the chip (i.e. <20 mm²), the yield achieved by hand assembly was poor since the elastomer was difficult to control, frequently unintentionally spreading across the electrode array or failing to insulate all the bondwires. Therefore this research sought to improve the prototype assembly methods.

An overview of the biosensor packaging market was presented in Section 1.5. It was explained that MEMS have driven the need for new forms of packaging [322] but there is a
scarcity of suitable solutions for cell-based biosensor ICs. Prototyping solutions have therefore been developed by researchers to meet specific needs. A method optimised by the Max Planck Institute for Polymer Research [71] uses a customised epoxy ring adhered between the sensor and bondpads before a potting resin or room temperature vulcanising (RTV) silicone elastomer is used to cover the bondwires. This approach has been further developed by [160] to extend lifetime up to at least three months by using EPO-TEK 302-3M (Epoxy Technology Inc., U.S.). These solutions appear to be well suited to ICs that have a fairly large die area compared to the sensor area (e.g. a die of 48 mm$^2$ with sensor area of 6.4 mm$^2$, as used by [172]), where the distance between the central sensor area (such as an array of microelectrodes) and the bondpads is large, e.g. greater than 2 mm. This allows for relatively easy / low tolerance placement of the epoxy ring. ICs with such geometries typically have amplifier and logic circuits in the area between central sensor and the bondpads at the IC periphery. Alternatively, [72] and [73] have developed packaging solutions based on SU-8 and Loctite photo-patternable adhesives where a thick coating (~1.5 mm) exposes the sensor area whilst leaving the bondpads and bondwires coated. These photo-patternable methods are also attractive due to their simplicity and have reported to be a repeatable assembly process for cell-based sensors with a short lifetime of up to 7 days. Beyond this timescale it is reported in [72] that the SU-8 suffers excessive electrical leakage. Similarly, in [73] it was found that the useful lifetime of the Loctite 3340 adhesive was one week and was incompatible with ethanol sterilisation.

In appraising the above methods for the proposed MEA design, it was anticipated that fabrication and placing a thin epoxy wall between the sensor and the bondpads would be prohibitively difficult with the small IC and sensor array geometries. Also, the above limitations of SU-8 and Loctite 3340 would be unsuitable for neuronal recording applications where the lifetime of the package must be considerably longer than one week. The following section therefore presents two new methods for assembly.

### 7.2 Materials and Methods

The ICs used to develop the assembly methods had first to be designed and fabricated via EUROPRACTICE.
7.2.1 IC Design

The design was to have the following specification:

i. An array of 48 electrodes
ii. Each pad connected directly and individually to a bondpad (one pad per pin)
iii. Circular electrodes, 30 µm diameter
iv. Use a minimal area of silicon to minimise cost.
v. Pads to have interlayer dielectric (ILD) directly below the pad metal layer
vi. Fabricated using the austriamicrosystems (AMS) 0.8 µm CXQ process

The choice of 30 µm pads was guided by the literature (e.g. [160]) and the commercial Multi Channel Systems MEAs (Section 2.9.5). Due to the ‘potential divider’ effect discussed in Section 2.5.2, it was considered that 10 µm diameter pads might be better suited to the diameter of mammalian neurons, but since the 30 µm pads of the Multi Channel Systems MEAs had already been proven it was decided not to risk changing the design specification.

The IC was designed using the Cadence Virtuoso Layout Suite (Cadence Design Systems Inc). A standard bondpad is shown in Figure 64 and an exploded view in Figure 65. The order of the layers in the schematic is not important and hence the exploded view shows the layers out of sequence with the physical process. The base of the bondpad is a diffusion area within the silicon followed by a ‘contact’ (an opening through the lower interlayer dielectric). Metal1 is then deposited onto this contact. A ‘via’ then opens a window in the second ILD to connect Metal1 to the following layer, Metal2. A window through the passivation down to Metal2 is defined by the ‘PAD’ mask. This standard bondpad was unsuitable for the electrode design as ILD was required below Metal2 (c.f. Section 5.2.3.5). The redesigned pad is shown in Figure 66. The MET2 is required to exceed the diameter of the pad by ≥7.0 µm to meet the layout design rules for this process [323]. This is highly significant to the proposed porous alumina process as this metal ring may provide reduced access resistance to the area undergoing anodisation and might lower the electrode operating impedance.

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57 Rule RE31, ‘Minimum MET2 enclosure of PAD’.
58 With no MET2 aluminium around the pad periphery, the resistance between the metal track entering the pad at one side of the pad and anodisation in progress at the opposite side of the pad is determined by the resistance of the remaining aluminium and titanium below the porous layer. The length of this resistance is the same as the pad width (i.e. the current must flow from one side to the other). However, with aluminium around the periphery, the maximum length is reduced to the pad radius.
Figure 64. Cadence Virtuoso schematic of a standard bondpad.

Figure 65. An exploded view of a standard CXQ process bondpad. From left to right: Metal 2 (MET2), diffusion, Metal1 (MET1), contact (CONT), via, passivation window (PAD).
The circular electrode pads were connected to bondpads via standard tracks. However due to the relatively large anodising currents, special attention was given to current density process parameters [324]: Metal2 maximum current density, $J_{MET2}(\text{max}) = 3.0 \, \text{mA} \cdot \mu\text{m}^{-1}$; Metal1 maximum current density, $J_{MET1}(\text{max}) = 0.9 \, \text{mA} \cdot \mu\text{m}^{-1}$. Two methods of minimising track current density were considered: firstly, by combining Metal1 and Metal2 and, secondly, by maximising the track width to the 30 µm of the pad.

With Metal1 and Metal2 in parallel, the division of current in each conductor is defined by the sheet resistances, $R_{MET1} = 70 \, \Omega \Box^{-1}$ and $R_{MET2} = 35 \, \Omega \Box^{-1}$. For a pad current, $I_{pad}$, the respective Metal1 and Metal2 currents are then $I_{pad}/3$ and $I_{pad}/2/3$. For this combined Metal1 and Metal2 configuration and a track width of 30 µm, the maximum current is then defined by the parameter $J_{MET1}$, giving a maximum $I_{pad} = (3 \times 0.9) \times 30 = 81 \, \text{mA}$. However, for Metal2 alone (without Metal1 in parallel), the maximum current in a 30 µm width track is defined by $J_{MET2}$, resulting in a maximum $I_{pad} = 3 \times 30 = 90 \, \text{mA}$. Therefore, for maximum current specification, it was decided to use only MET2 for the tracks connecting the electrodes to the bondpads.
The peak anodising current, $I_{pk}$, for the 30 µm diameter pad was calculated in Section 5.2.3.1.1 to be 177 nA. The minimum Metal2 track width, $w$, is then defined by JMET2 (max) giving $w_{(min)} = JMET2 \times I_{pk} = 3.0 \times 0.177 = 0.53$ µm. The design therefore only required the minimum track width of 1.2 µm, but to allow processing flexibility it was decided to use tracks 20 µm wide (the maximum width allowed without slotting). The maximum current specification of the pads is therefore 60 mA, which provides an excellent margin to requirements.

7.2.2 Assembly

The first method chosen for development built upon the experience at King’s College London using the biocompatible elastomer Silastic 9161 (Dow Corning, UK), but was to be improved by using a ‘mould-based process’ to overcome the difficulties of elastomer placement. A second packaging solution based on the commercial ‘partial encapsulation’ process provided by Quik-Pak (San Diego, U.S.) was also investigated. It was believed that this was the first time this commercial process had been adopted for a cell-based biosensor application and therefore its biocompatibility was unproven.

For assembly, the ICs fabricated according to Section 7.2.1 above were used. Thirty devices were supplied in 48-lead ceramic dual-in-line packages (DIP) with removable die-cavity lids. A further 20 dice were supplied unpackaged.

For all devices, a 10 mm tall glass cylinder culture chamber (QB Glass, UK) was to be adhered to the top of the ceramic package so that it encircles the open die cavity. A cyanoacrylate adhesive was used for a permanent bond, but generally the glass was adhered using Silastic 9161 (Dow Corning, UK) so that the packages could be more easily disassembled for SEM analysis of the processed electrodes.

7.2.2.1 Mould-based Assembly

The principle of the proposed mould-based process was to initially shield the sensor array with a water-soluble mould. An RTV elastomer was then to be applied over the whole chip so that it filled the cavity, covering bondwires and bondpads. The water-soluble mould was then dissolved to leave the exposed sensor electrodes.
To form the water-soluble mould, a reusable aluminium mould template was prepared using basic machine-shop tools (Figure 67). The critical dimension is the size of the aperture at the base of the mould. Above the aperture, a conical taper was formed using a 45° countersink bit – the resulting open well shape is preferred for good cell plating, ease of microscopy and possibly better diffusion of media into and out of the well. The angle must be sufficient to ensure that the well sides cover the knee of the bondwires where they rise away from the bondpads. The mould itself was formed of polyethylene glycol (PEG) with average molar mass of 35,000 g·mol⁻¹ (Sigma-Aldrich, UK), supplied as flakes a few millimetres in length. The mould was formed by placing the aluminium template on a glass microscope slide, heated on a hotplate to approx 100°C and then flakes of PEG were melted into the mould. A solid core wire ‘handle’ was then inserted into the mould and held in position with cross-grip tweezers whilst the PEG was allowed to cool. By using a template of two halves, the mould (Figure 68a) can more easily be released. Fillets of excess PEG, resulting from the template joint, were removed with a modelling knife.

![Figure 67. a. The reusable aluminium mould template (Minor scale units at bottom of the image are 1 mm); b. Side view of half mould. The die aperture (width 1.6 mm) is at the top of the image. The inset shows the same view but with the mould outline highlighted.](image-url)
Preliminary experiments found that the solid PEG-35,000 does not form a seal with the sensor surface that is sufficient to keep out the fluid elastomer. This was resolved by applying a thin layer of waxy PEG to the centre of the mould base (Figure 68b). As the mould was lowered onto the IC surface the waxy PEG was squeezed and formed a tight interface. It was found that a 1:1 weight ratio of PEG-1000 and PEG-1450 (Sigma-Aldrich, UK) was a suitable formulation, where 1000 and 1450 are the respective average molar masses (g·mol⁻¹). PEG was chosen due to its good biocompatibility and its low melting temperature (~64°C for the Sigma-Aldrich PEG-35,000). This enabled easy removal of the mould by melting at a temperature that is also compatible with the cured elastomer.

7.2.2.1 Mould-based Assembly Process

No specific equipment was necessary for the PEG process, but the mould had to be positioned accurately over the sensor array during the application and curing of the elastomer. This required some form of three dimension micro-manipulator: an optical bench was used. The following is the mould-based process for assembling the MEA:

1. Adhere a glass culture chamber to the top of the IC package with exposed die cavity.
2. Apply waxy PEG to base of the mould. Remove excess waxy PEG from the periphery of the PEG mould with a modelling knife.
3. Lower the mould into position on the die surface, causing the waxy PEG to be squeezed to form a tight interface.

4. Fill the die cavity with Silastic 9161 RTV elastomer (drip from the end of a wire).

5. Allow the elastomer to cure (~4 hrs).

6. Submerge the IC in a dish containing de-ionised water and place in an oven at ~80°C for 15–20 minutes until the PEG mould melts and dissolves (indicated by the wire handle falling from its vertical position).

7. Remove the remnants of the PEG mould by jetting with water at ~80°C using a disposable pipette.

8. Dry the package using a compressed air line (air duster).

9. Inspect for a clean surface. Repeat steps 7–8 until the sensor well is clear of PEG.

7.2.2.2 Partial Encapsulation Assembly Process

The principle of the proposed partial encapsulation method was to adhere a frame to the IC surface to define the sensor window, then back-fill the void behind the frame with mould compound to cover the bondwires.

The bare dice were shipped to Quik-Pak (San Diego, U.S.) for partial encapsulation. Their standard manufacturing process was used except that biocompatible compounds were specified: Silastic Medical Adhesive Silicone Type A (Dow Corning, U.S.) for placement of the window frame and Hysol CB064 (Loctite, U.S.) for encapsulation. The following is a summary of Quik-Pak’s process:

1. Select an open-cavity package with suitable lead frame to complement the size of the die.

2. Die-attach (to bond the die to the lead-frame). A conducting die-attach was used, but non-conducting die-attach can also be specified, depending on the IC substrate biasing requirement.
3. Wire bond (i.e. attach bondwires between lead-frame and IC bondpads).

4. Design and laser cut the epoxy window frames. The frame wall thickness used was only 0.13–0.15 mm. The frame height can exceed the final IC height as the frame is milled flush at step 9.

5. Attach the frame to the die using a low-stress epoxy (e.g. Silastic Medical Adhesive Silicone Type A).

6. Epoxy cure (72 hours at room temperature).

7. Back-fill the void with moulding compound (Hysol CB064) to cover the bondwires.

8. Mould compound cure (2–3 hours at 110ºC followed by 2–3 hours at 150ºC).

9. Mill the top of the package to planarise the package, the mould compound and the frame, and also to minimise the height of the cavity.

10. Remove any milling debris using a compressed air duster.

After completion of the Quik-Pak process, the devices were finished by adhering the glass culture chambers using Silastic 9161 elastomer.

### 7.2.3 CMOS Electrodes Post-Processing

Packaging biocompatibility tests were performed after development of the electrodes had progressed. All devices were post-processed as discussed in Chapter 9. Briefly, this entailed anodisation of the aluminium electrodes for approximately 40 minutes in 0.4 M phosphoric acid, followed by 20 minutes plating in a 59 mM gold chloride H.Au.Cl₄.3H₂O bath and approximately one minute for platinum black deposition using chloroplatinic acid (CPA) H₂PtCl₆.6H₂O with 264 µM Lead(II) acetate trihydrate.

### 7.2.4 Cell Culture

Experience at King’s College London using Silastic 9161 elastomer for packaging had previously established its biocompatibility [326],[327]. However, the Quik-Pak process used materials new to cell culture and therefore required an evaluation of biocompatibility. The
choice of the NG108-15 mammalian neuronal cell line for these tests was governed by the proposed applications, ease of use and past experience. Cells were cultured as described in Section 5.3.1. Sterilisation of the ICs was performed in the laminar flow hood by submersing in ethanol for 30 minutes followed by air drying for approximately 15 minutes. The ICs were coated with poly-l-lysine (poly-l-lysine hydrobromide 15–30 kDa, 0.01% w/v (P7890, Sigma-Aldrich, UK)), incubated for 1 hour at 37°C, then rinsed with growth medium. The packaged ICs were plated with cells (seeding density of 30,000–60,000 ml⁻¹) and incubated at 37°C, 10% CO₂. Growth medium was replaced after 24 hours with differentiation medium (DMEM with GlutaMax-II, 1% FCS, HT supplement (30 μM hypoxanthine, 4.8 μM thymidine, Sigma-Aldrich), 10 μM prostaglandin E1, 50 μM IBMX in 50 mM DMSO, Penicillin-Streptomycin as previously) and replaced after a further 24 hours with plating medium (as differentiation medium but excluding the prostaglandin E1 and IBMX). The cells were then incubated for a further 13 days, refreshing the plating medium every 3–4 days, prior to an evaluation of cell vitality. Biocompatibility was tested by one-way ANOVA (Analysis of Variance) with a null hypothesis (α < 0.05) that the Quik-Pak partial encapsulation devices have the same number of cells per unit area as the devices assembled using the mould-based process.

7.3 Results and Discussion

7.3.1 IC Design

The ICs were successfully fabricated by AMS, as illustrated by the schematic of Figure 69. A single IC was sacrificed to confirm the pad windows had been correctly defined: a drop of silver paint (Electrolube, UK) was placed over several pads and a multimeter used to measure the resistance between the pads via their respective package pins. The low resistance (<1 kΩ) verified correct operation of the ICs.
Figure 69. The completed ‘WET4’ die schematic. For context, the figure includes a photograph of an old CMOS IC assembled by manual application of the Silastic 9161 elastomer. The central void in the elastomer contains the sensor shown in the schematic. The side dimension of this square IC is 3.16 mm. The central area of the IC is the array of 48 circular electrodes of 30 µm diameter. The square bondpads are at the periphery of the IC. The width of the electrode array is 1.2 mm with 0.7 mm between array and bondpads.

7.3.2 Assembly

The devices assembled using the PEG mould-based method were subsequently anodised. The application of the 60 V anodising potential to the pads using a Keithley 3200 SourceMeter was also used to detect any excessive electrical leakage between bondwires or bondpads to the electrolyte in the culture chamber.\(^59\) No such problems were noted with the exception of one assembly defect (see below), demonstrating that the elastomer insulation was functioning

\(^59\) Typical array anodising currents were <2 nA. Excess leakage was simply a current >> 2nA. The limit of the test was 100µA, as set by the supply.
correctly (Input pin leakage, $I_{in\text{ (typical)}} < 2 \text{ nA at } V_{in}=60 \text{ V})$. Disassembly of a device showed a good width ($\geq 200\mu\text{m}$) of elastomer between well and bondpads: Figure 70a shows the location of the elastomer removed from the die which was flipped over to give the underside view (Figure 70b). One electrode in the array of 48 was almost lost due to creep of elastomer under the mould which was probably due to the mould not being lowered square to the die surface: this confirms that the placement of the waxy PEG and the lowering of the mould require care. A further device did fail leakage tests which analysis showed was due to misplacement of the PEG mould causing the bondpads to be exposed to the sensor well. The resulting yield was $28 / 30 = 93\%$. Although not attempted, the process does allow an IC to be reworked where a mould is incorrectly placed: the mould can simply be lifted and the die cleaned of PEG in hot water. Such rework is not possible with an epoxy ring process.

Figure 70. Disassembled elastomer removed from between the bondpads and the sensor well. Image (a) is only to assist with orientation, showing part of the elastomer overlaid onto the IC schematic. This elastomer was removed from the die and flipped over to give the underside view (b) which is therefore looking upwards from where the die was positioned. During assembly, the PEG mould would have filled the void (i) on the right side of the image which is the sensor well opening. Detached bondwires (ii) are visible on the left hand side of the image and indicate the location of the bondpads. A cavity (iii) has been created in the elastomer where waxy PEG had extruded from the sides of the mould when lowered onto the die. The elastomer between the cavity and the bondpads provides electrical and chemical isolation: the minimum measured width (~200 µm) is highlighted (iv).

It can be seen from Figure 71 that during application of the moulding compound a meniscus is formed around the PEG mould. This left a ‘spout’ at the top of the well but was thought unlikely to have an effect on the cells cultured within the well. Attempts to modify the
properties of the elastomer, such as by addition of 10 wt% silicone fluid, did not reduce the meniscus. It may be possible to eliminate it by a second application of elastomer to top-up its level to the lip of the spout, but this was not attempted.

An initial concern of using PEG was that it can modify a surface and reduce cell adhesion [328]. However, the PEG was dissolved during the assembly process and the die surface underwent considerable further processing in the anodising acid and plating solutions which should have ensured the PEG was removed. Additionally, the aluminium electrode surfaces were modified by the anodisation and plating processes and so any remaining PEG monolayer should have been removed by these steps.

Devices assembled using the Quik-Pak partial encapsulation method were similarly tested and also found to be electrically functional. The yield was 90% (18/20) although this was considered worst-case since the two initial failures could be considered as process setup samples. The partially encapsulated devices had consistent and regular windows (Figure 72)
and avoided the need to clean the sensor array of PEG. This was therefore the preferred solution since, firstly, it leverages the process quality typically achieved on a commercial manufacturing line, and secondly, the total packaging cost for 18 dice (£1100) was comparable to having the die attached and bonded into the ceramic packages (£880) after which manual elastomer assembly is still required. It is appreciated that plastic packages are not hermetic and so further evaluation may be required for sensor applications where absorption of water and ions into the moulding compound are a concern.

After 14 days in culture on the biosensors, cell vitality tests \((n \geq 11)\) showed significantly \((p \leq 0.001)\) more cells on the Quik-Pak devices versus the devices assembled with the Silastic 9161 elastomer (Figure 73). Visual inspection of the devices showed no evidence of compound incompatibility or corrosion problems. The reason for the improved vitality on the Quik-Pak devices was not clear, but three differences were noted: firstly, the use of PEG as a mould; secondly, the shape and size of the sensor well; and thirdly, the Silastic 9161 was not present on devices assembled by partial encapsulation. As previously mentioned, the PEG was likely to have been removed during the CMOS post-processing (anodisation and plating.

![Image](image_url)

Figure 72. A completed biosensor assembled using partial encapsulation by Quik-Pak. The tracks and array of the WET4 IC can be seen in the well. Adhesion of the glass culture chamber was the only manual assembly step. (The outside diameter of the glass ring and the package width is ~14 mm.)
of electrodes) followed by subsequent ethanol sterilisations and poly-l-lysine coatings. The shape of the well formed with the mould-based process would be expected to increase the number of suspended cells guided to the IC sensor rather than decrease the number since the elastomer meniscus formed a funnel. This was therefore inconsistent with the observation. The final factor was the packaging compound, which seemed to be the most likely explanation for the observed differences in cell vitality, but would require further work to confirm this.

Figure 73. NG108-15 cell density after 14 days in culture on the biosensors assembled with the mould-based process (mean, $\bar{x} = 1280$ mm$^{-2}$, standard deviation, S.D. = 1054 mm$^{-2}$, $n = 11$) and the partial encapsulation process ( $\bar{x} = 7283$ mm$^{-2}$, S.D. = 5445 mm$^{-2}$, $n = 14$). These results show there were significantly more (p < 0.001, Welch’s t-test) cells on the Quik-Pak partial encapsulation devices. Error bars represent 95% confidence intervals with markers indicating means.
7.4 Conclusion

Two new assembly methods have been developed for prototype assembly of biological IC sensors which meet the requirements for a lifetime of eight weeks. The method of manual encapsulation of bondwires using a PEG mould-based process and the method using commercial partial encapsulation by Quik-Pak both yielded functional sensors. Cell vitality tests showed the partially encapsulated devices were biocompatible, with cell densities exceeding those of the devices assembled with the Silastic 9161 RTV elastomer. The Quik-Pak process, available for DIP and other package outlines, provides an efficient solution to the problem of assembly of biological sensors and avoids the precision manual processing required when assembling these devices in the laboratory. The observed biocompatibility demonstrates that the partial encapsulation process may be suitable for commercial manufacture of cell-based biosensor ICs.

Key Points

- Packaging methods suited to cell-based biosensors are scarce.
- Two new prototype biosensor packaging techniques have been developed and demonstrated to be biocompatible.
- The IC design provides additional verification that CMOS tracks have excellent margin to the current densities required for anodisation.
8 Barrier Oxide Processing and Real-Time Impedance Measurement

8.1 Introduction

From Section 3.2.2 and Figure 32 it is recalled that the proposed electrode design is dependent on conductivity from the top of the alumina surface, through the barrier oxide, to the titanium layer. This layer connects to the periphery of the electrode pad where the standard aluminium CMOS track is connected to an amplifier circuit for, say, neuronal recording applications, or a driver circuit for neuronal stimulation. It was explained that reducing the impedance of the barrier oxide was expected to be a key aspect of the design.\(^{60}\)

From a perspective of electronic engineering, semiconductor manufacturing and materials science, the basic understanding of an anodised film is that it is electrically insulating. Indeed, alumina can be used as the dielectric in capacitors [223]. The processing of porous alumina to make it conductive is a specialist field and presently remains in the realm of academic research. Processing will be reviewed below and forms the basis for the experimental work of this section. It will be shown that the application of these specialised processing techniques is pivotal to the correct function of the proposed electrode.

Additional processing and analytical capabilities were required before further progress on electrode development could be made. Firstly, it was necessary to measure the impedance of the plain aluminium and unmodified anodised films. Secondly, to process the porous alumina in a manner that reduced the impedance at the barrier oxide. Thirdly, to configure instrumentation for these measurements.

The established technique to characterise the impedance of a wet electrode (i.e. at a solid-solution interface) is electrochemical impedance spectroscopy (EIS). Briefly, an a.c. waveform is applied across an electrochemical cell containing the working electrode (the

\(^{60}\) The required reduction in impedance is determined by application, but it can be considered that the anodic barrier oxide impedance is sufficiently low when it no longer dominates the equivalent circuit. i.e. it will be shown in this and subsequent chapters that the electrical double layer at the solid-solution interface will dominate if the barrier oxide impedance is reduced. For a planar metal electrode, the target impedance was therefore set as that of the unmodified aluminium pad.
electrode under test), an electrolyte and a counter electrode. The resulting a.c. current is measured whilst sweeping frequency. The comparison of applied a.c. voltage versus the measured a.c. current’s amplitude and phase enable the complex impedance to be plotted as a function of frequency. Further background on EIS is available in [329]. EIS is an extremely powerful technique enabling the identification of many physical electrochemical mechanisms. Additionally, equivalent electrical circuits can be fitted to the impedance data to allow simulation of electrodes. This was to be of direct benefit to this development work since EIS data from processed coverslips could be used to model CMOS electrodes.

8.1.1 Barrier Oxide Impedance

An outline of potential methods for reducing the barrier impedance was presented in Section 3.2.2.1. The literature review did not identify experimental conditions that were directly applicable to this work (i.e. for CMOS film composition) and therefore further research was required to evaluate the four identified methods to reduce impedance:

i. deformation of the pore base when anodising a thin film down to a conductive substrate
ii. a chemical etch to thin or remove the barrier oxide at the pore base
iii. electrochemical thinning of the barrier oxide by ramping the anodising current or voltage
iv. reduction of the barrier oxide’s electronic band gap by introducing defect levels from metal ion species, e.g. during anodisation or by electrodeposition.

8.2 Methods

Initial experiments were performed using the apparatus detailed in Section 5.2.2 (based on the Keithley 236 Source-Measure Unit and Labview). The majority of experiments were later performed based on the Agilent 4294A Precision Impedance Analyzer. Stand-alone EIS was performed using a Solartron 1260A Impedance/Gain-phase Analyzer with 1296 Dielectric Interface. The methods are summarised in the text and full details for each sample provided in Appendix B.

For EIS, it was necessary to devise a custom interface to the coverslips. A suitable EIS rig was designed (Figure 74) and is illustrated in (Figure 75). To enable the resistance of the
electrolyte to be calculated, the dimensions of the void between the flat bottom of the brass counter electrode and the working electrode area formed by the O-ring circular area were determined from the design. The resistance, $R$, for each segment of the volume (in series) were calculated based on $R = \rho \cdot l / A$, where $\rho$ is the known resistivity of a 10% w/v $\text{K}_2\text{SO}_4$ electrolyte and $A$ is the area of the electrolyte in each segment. This was to be used to validate EIS data.
8.3 Materials

Initial experiments were performed on glass coverslips with ~40 nm of titanium followed by a deposition of approximately 960 nm of pure (>99.5%) aluminium (See Section 5.2.2). Later experiments were performed with similar substrates except Al–1.0 wt%Si–0.5 wt%Cu was used. Unless otherwise stated, anodisation was performed using a 0.4 M (4 % w/v) phosphoric acid electrolyte at 21 ± 2°C. For economic use of the coverslips, approximately ⅔ of the length was anodised (the top third being used to connect to the crocodile clip) and the bottom ⅓ plated. Each coverslip therefore provided a plated area with porous alumina and aluminium controls in the same sample. Coverslip serial numbers were abbreviated to ‘Slip x’.

8.4 Preliminary Barrier Oxide Conductance Tests

These initial experiments evaluated the capability of the Keithley 236 to ramp voltage and current and to attempt platinum electrodeposition using a chloroplatinic acid (CPA) bath.

8.4.1 Methods

Coverslips were anodised either to ~⅓ the film thickness (determined by anodising time) or fully anodised until the steady state current reduced by a factor of either 10 or 40. Oxide
thinning was also attempted by ramping the anodising voltage down from 30 V to either 10 V or 6 V. The CPA bath was 24 mM (1%) H$_2$PtCl$_6$·6H$_2$O (Sigma-Aldrich, UK) with 264 µM (0.01%) Lead(II) acetate trihydrate, operated at 45°C. Samples were analysed using the SEM and FIB of the dual-beam Carl Zeiss 1540XB system (See Section 6.4.2). EIS data were analysed using ZView v.3.1 (Scribner Associates Inc).

### 8.4.2 Results and Discussion

Visual inspection of the substrates suggested they had correctly anodised: fully anodised coverslips appeared almost transparent (due to the transparent porous alumina layer and only a thin 40 nm titanium layer); partially anodised coverslips remained opaque. Platinum deposition was visible on Slips 4–6. SEM analysis confirmed the complete anodisation (Figure 76). There did not appear to be a difference in barrier oxides between coverslips that were partially anodised versus fully anodised: all had barrier oxides of ~50 nm under the porous layer (Figure 76). The shape at the pore bases could not be resolved on these samples anodised at 30 V. Fully anodising a coverslip at 100 V showed a deformation of the pores bases similar to [233] but no defects were visible at the edges of the inverted oxide (Figure 77, c.f. Figure 33d). Deposition of platinum was confirmed (Figure 78) but showed that the alumina layer had been unintentionally removed by the deposition process. The platinum also appeared porous (as in platinum black) suggesting the deposition had been too fast (i.e. the current density was too high). The CPA bath had been operated at standard industrial conditions according to [330], with 45°C operation being chosen as the lowest recommended bath operating temperature. However, an unbiased dip-test (CPA, 45°C, 10 mins) demonstrated that aluminium and alumina were etched by this bath. This was believed to be due to the bath being very acidic (pH 0). Aluminium and alumina are known to corrode at pH 0 (Figure 13) and so an alternative plating bath was sought.
Figure 76. Preliminary anodisation, deposition, and imaging experiment results (Slip 2, porous alumina anodised at 30 V to $I_{\text{anod.}}/40$). Approximate thicknesses of porous layer (H1), barrier oxide layer (H2) and titanium layer (H3) are marked. Contrast and resolution were limited due to the insulating properties of the specimen (i.e. only the thin titanium layer was conductive).
Figure 77. Anodisation to completion and at high voltage (100 V) to resolve the shape of the barrier oxide at the pore bases. The oxide is inverted within the centre of the pore bases with voids between the oxide and the titanium layer. No crack defects similar to those observed in [233] (Figure 33d) are visible across the oxide. Scale bar is 200 nm.
Figure 78. Platinum deposition was confirmed (Slip 4), with a film thickness of approximately 2 µm. Unfortunately the alumina layer had been etched – it could not be determined if the titanium layer was still present or whether the platinum was directly on the glass substrate. Additionally, the platinum appeared to be porous (i.e. platinum black).

Figure 79 shows impedance magnitude, \(|Z|\), per unit area and phase, \(\phi\). Unprocessed aluminium showed a predominantly capacitive characteristic (i.e. \(\phi < -50^\circ\) except at low frequencies) which is due to the double layer capacitance (Section 2.3.1). This is usually considered a ‘constant phase element’ due to the phase being somewhat larger than \(-90^\circ\). Anodising to form porous alumina resulted in the film becoming more capacitive (\(\phi \rightarrow -90^\circ\)) with very high impedance at low frequencies. The phase indicated the substrate becomes less dominated by the double layer capacitance after platinum deposition, as shown by the significant resistive element for slip 4. These data illustrate the objective of the barrier oxide thinning: the requirement was to reduce the impedance for the porous alumina to have the characteristics of the platinum (black) surface (but without etching off the porous layer that prevents corrosion). Equivalent circuits for the interface are shown in Figure 80. Data can be fitted to these models using Zview, as was performed in later experiments.
Figure 79. EIS data (Solartron 1260A/1296, room temperature) for initial porous alumina (anodic aluminium oxide (AAO), plated and aluminium controls. At $10^{-2}$ Hz, the aluminium has $|Z| = 2.2 \times 10^5 \Omega$ and the porous alumina $4.8 \times 10^7 \Omega$, a factor of 218 difference. Plating reduces the impedance to $7.5 \times 10^3 \Omega$, a factor of 6400 with respect to the porous alumina and a factor of 29 lower than the aluminium. Area = 0.5 cm$^2$.

Figure 80. Equivalent circuits for fitting to EIS data: a. ‘Randles Cell’ [331] for a solid-solution interface; b. Randles Cell with additional components for porous alumina to represent the barrier oxide impedance. $R_s$ is the solution resistance (i.e. the electrolyte in the void between the substrate and the brass electrode of the test rig), $R_{ct}$ and $CPE$ are the respective resistive and constant phase elements of the double layer, $R_b$ and $C_b$ represent the impedance of the barrier oxide.

8.4.3 Conclusions (of Preliminary Conductance Tests)

These preliminary tests showed the barrier oxide increases the low frequency ($10^{-2}$ Hz) impedance by a factor of ~200 times which illustrate the need to reduce this impedance by further processing. Platinum plating was demonstrated, but damaged the porous alumina layer due to the corrosive action of the CPA bath. This juncture lead to two paths of investigation:
firstly, thinning the barrier layer to form a plain porous alumina electrode and, secondly, improving electrodeposition. However, since the high impedance of the barrier could have been a cause of lack of nucleation of platinum in the pores, both aspects of the work were progressed in parallel: i.e. coverslips showing an improvement in barrier oxide thinning could be tested for improved deposition. Further, the two lines of investigation were linked by the possibility of reducing the barrier oxide’s electronic band by electrodeposition.

8.5 Platinum P-Salt Bath

Since the preliminary experiments with the platinum CPA bath had caused etching of the porous alumina, a neutral (pH 7) ‘P-salt’ bath was tested.

8.5.1 Methods

The P-salt bath was prepared in accordance with [332], comprising 16 mM (5.0 g l\(^{-1}\)) dinitrodiammine-platinum Pt(NH\(_3\))\(_2\)(NO\(_2\))\(_2\), (Strem Chemicals, UK) in a buffer of 674 mM disodium hydrogen phosphate dihydrate and 136 mM diammonium hydrogen phosphate, pH adjusted to 7.0 using NaOH or H\(_3\)PO\(_4\).

8.5.2 Results and Discussion (of P-Salt Bath)

‘Dip tests’ performed at the minimum specified bath operating temperature of 89\(^\circ\)C resulted in rapid etching of the porous alumina and aluminium films. Additional dip tests showed this corrosion could be prevented by reducing the temperature to below ~50\(^\circ\)C. Films tested at 21\(^\circ\)C and 45\(^\circ\)C appeared stable at 30 mins. Subsequent P-salt depositions were performed at 35\(^\circ\)C to provide margin to the corrosion problem. To understand further the cause of corrosion additional substrates were tested in the P-salt buffer solution (i.e. without the platinum salts) at 89\(^\circ\)C. This caused corrosion indicating the buffer was responsible for the problem and not the dinitrodiammine-platinum.

8.6 Improved Barrier Thinning

The preceding FIB specimens had shown a thick (~50 nm) barrier oxide on all samples, causing the high substrate impedance. Barrier thinning has been successfully achieved elsewhere: a review is provided in Table 11.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Substrate</th>
<th>Barrier Thinning Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mei et al. (2006) [333]</td>
<td>Al substrate, partially anodised</td>
<td>Voltage ramp, ~1 Vs⁻¹</td>
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<tr>
<td>Kim et al. (2006) [334]</td>
<td>Al on 250 nm Ti</td>
<td>Chemical etch</td>
</tr>
<tr>
<td>Sauer et al. (2002) [335]</td>
<td>Al substrate, partially anodised</td>
<td>Chemical etch + current steps (halving) to ~6 V</td>
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<tr>
<td>Sklar et al. (2005) [336]</td>
<td>Al substrate, partially anodised</td>
<td>Chemical etch + re-anodise at 25 V + current ramp to 6 V</td>
</tr>
<tr>
<td>Nielsch et al. (2000) [337]</td>
<td>Al substrate, partially anodised</td>
<td>Chemical etch + current steps to 6-7 V</td>
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<tr>
<td>Wu et al. (2004) [338]</td>
<td>Al on silicon substrate</td>
<td>Chemical etch</td>
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<tr>
<td>Forrer et al. (2000) [339]</td>
<td>Al substrate, partially anodised</td>
<td>Voltage steps</td>
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<tr>
<td>Montero-Moreno et al. (2008) [340]</td>
<td>Al substrate, partially anodised</td>
<td>Current steps (various and optimised schemes) to &lt;10 V</td>
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<td>Zhou et al. (2008) [341]</td>
<td>Al substrate, partially anodised</td>
<td>Voltage steps down to 2 V</td>
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<tr>
<td>Saedi and Ghorbani (2005) [342]</td>
<td>Al substrate, partially anodised</td>
<td>Constant current to 18 V + constant 18 V</td>
</tr>
<tr>
<td>Tian et al. (2005) [343]</td>
<td>Al substrate, partially anodised; Al on Ti / SiOₓ / Si substrate</td>
<td>Reverse bias (-3 V to -5 V) in sulphuric electrolyte (time controlled)</td>
</tr>
<tr>
<td>Xu and Huang (2008) [344]</td>
<td>Al substrate, partially anodised</td>
<td>Voltage ramp to 5-6 V + constant 5 V.</td>
</tr>
<tr>
<td>Salka et al. (2007) [236]</td>
<td>Al substrate, partially anodised</td>
<td>Constant current; current steps</td>
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<tr>
<td>Evans et al. (2006) [345]</td>
<td>Al on 5 nm Au on 20 nm Ta on Si</td>
<td>None (Anodised to Au layer)</td>
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<tr>
<td>Rabin et al. (2003) [346]</td>
<td>Al on 40 nm Ti, 50 nm Pt on Si; Al on 250 nm Ti on Si substrate</td>
<td>None (Anodised to Pt layer) Reverse Bias (potassium chloride, -2.25 V)</td>
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<td>Crouse et al. (2009) [347]</td>
<td>Al on 3 nm Ti on 30 nm Pt on 7 nm Ti on Si substrate</td>
<td>Pulsed reverse and forward bias</td>
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<td>Pruneanu et al. (2000) [348]</td>
<td>Al substrate, partially anodised</td>
<td>Chemical etch</td>
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<tr>
<td>Mori et al. (2008) [349]</td>
<td>Al on Au/Ta on Si substrate</td>
<td>Chemical etch</td>
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<td>Al substrate, partially anodised</td>
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<td>Al on 250 nm Ti on Si substrate</td>
<td>Chemical etch</td>
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<td>Al on 120 nm ITO on glass substrate</td>
<td>Chemical etch</td>
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<tr>
<td>Jeong et al. (2007) [354]</td>
<td>Al substrate, partially anodised</td>
<td>Constant current; current steps</td>
</tr>
<tr>
<td>Yasui et al. (2003) [355]</td>
<td>Al on 50 nm W or 20 nm Pt on 7 nm Ti on Si substrate</td>
<td>Chemical etch or WOₓ barrier penetration</td>
</tr>
<tr>
<td>Crouse et al. (2005) [356]</td>
<td>Al on Si substrate, or Al on Ta₂O₅ / Ti on Pt on Si substrate</td>
<td>Voltage ramped up (Ta/Ti layer electrical breakdown).</td>
</tr>
</tbody>
</table>

Table 11. Publications detailing alumina barrier oxide thinning.
The literature in Table 11 confirms that thinning of the barrier oxide is key, and is often performed either by a pore-widening etch or electrochemical thinning. The thinning technique and physical mechanisms are characterised in [340]. The initial current ramp tests of Section 8.4.1 were therefore to be developed further in this experiment. However, the CMOS metallisation presents a unique configuration of layers and it was unclear whether the barrier oxide could be thinned once the aluminium had anodised down to the titanium, or whether the voltage/current ramp should be initiated whilst aluminium remained below the barrier oxide.

Additional problems arose due to the high cost of the platinum solutions and the difficulty operating the experiments with only 10–20 ml of plating bath. For example, a plan to increase the bath concentration was not possible due to the prohibitive cost of the platinum. Since in several articles in Table 11 (e.g. [345],[337],[341],[354]) nickel had been successfully deposited into alumina pores, it was decided to temporarily change to nickel deposition, with the plan to revert back to platinum once the electrochemical and deposition processes were better understood. It should be stressed that there was no intention of using these nickel-coated substrates for biological experiments as the substance is cytotoxic. The plan was to revert to platinum deposition once the basic operating parameters had been defined using nickel, and then to use only platinum electrodes for the cell culture experiments.

### 8.6.1 Methods

Anodisation was performed as before. Termination schemes for the anodisation were defined as ‘partially anodised’, ‘to cusp’ and anodised to ‘completion’. With reference to Figure 37, partially anodised meant ceasing anodisation whilst part way through period c (where the current is defined as $I_{\text{steady}}$): this would therefore leave aluminium below the barrier layer. The ‘cusp’ was defined as the point at which the anodisation current was first detected to fall from the steady state, caused by the pores reaching the base of the aluminium (region d). The decrease in current is not instantaneous since not all pores reach the base of the layer at the same instant. Anodised to completion (fully anodised) was defined as the start of region e, where only a leakage current remains. Barrier oxide thinning was performed using galvanostatic anodisation, either $I_{\text{steady}}/10$ and terminating when the anodising potential had fallen to 10 V, or three galvanostatic steps (‘I steps’) consisting of $I_{\text{steady}}/2$ until $V = 15$ V (and measuring $I = I_{\text{steady}2}$) followed by anodising at $I_{\text{steady}2}/2$ until $V = 7.5$ V (and measuring $I = I_{\text{steady}3}$), followed by anodising at $I_{\text{steady}3}/2$ until $V = 5.0$ V.
A nickel ‘Watts Bath’ was prepared (30% w/v NiSO\(_4\)·6H\(_2\)O, 4.5% w/v NiCl\(_2\)·6H\(_2\)O, 4.5% w/v H\(_3\)BO\(_3\)) and an ‘RCT Basic’ hotplate and stirrer with temperature probe (IKA GmbH, Germany) was used to maintain accurately the bath operating temperature (35°C). A pulsed deposition scheme similar to that used in [337] and elsewhere was used, as it is stated that a pulsed scheme improves deposition. Briefly, the reasons for using pulsed electrodeposition are stated as, firstly, a large current pulse ensures that plating occurs at the base of the pores, despite any impedance due to remaining barrier oxide. Secondly, a reverse pulse quickly depolarises the system allowing replenishment of metal ions into the pores from the bulk solution. This scheme was generated for this and subsequent experiments by developing the custom circuit of Figure 81 and using an Agilent 33220A Function / Arbitrary Waveform Generator as a trigger to allow the period to be adjusted. This resulted in the desired 8 ms positive (cathodic) 70 mA plating pulse and 2 ms reverse bias (anodic) pulse of −3 V, followed by a rest period at 0 V. The default period set using the 33220A was 500 ms. The assembled circuit is shown in Figure 82. The correct operation was checked using an oscilloscope with the potential across a 1 \(\Omega\) series resistor (Figure 83). The arrangement of apparatus is shown in Figure 84.
Figure 81. Pulsed electrodeposition (PED). The circuit generates an 8 ms 70 mA ($I^+$) cathodic pulse, followed by a -3.0 V ($V^-$) 2 ms anodic pulse. Each cycle is triggered by the external Agilent 33220A waveform generator connected to PAD5, its period, typically 100-500 ms, defining the duty cycle, e.g. 8 ms/500 ms = 1.6% duty. The duration of $I^+$ trimmed by R7, $I^-$ trimmed by R8 and the amplitude of $I^+$ trimmed to 70 mA by R11. Capacitor C4 acts as a differentiator to trigger IC1 B. Relay K1 on the output stage allows optional connection to the Agilent 4294A for EIS readouts, after an iBasic program disables the PED circuit.

Figure 82. The assembled circuit for pulsed electrodeposition.
Figure 83. Oscilloscope waveforms from pulse generator circuit. The upper trace shows the potential, $V$, and the lower trace shows the current, $I$, through a $1 \, \Omega$ resistor ($1 \, \text{mV/div} = 1 \, \text{mA/div}$). The voltage trace starts at OCP prior to the 70 mA pulse. The 8 ms 70 mA pulse is followed by a $-3 \, \text{V}$ pulse. After completion of the $-3 \, \text{V}$ pulse the potential across the electrode gradually returns to the OCP.
8.6.2 Results and Discussion

Substrates were successfully anodised and plated. Visual inspection of the substrate anodised to completion (slip 15) showed that after electrodeposition the sample remained transparent suggesting little or no deposition. However, imaging with an optical microscope showed only a subtle change (Figure 85a). Improved nickel deposition was shown on slip 16 (anodised to cusp) and slip 17 (partially anodised). Nickel deposits on slips 16 and 17 were not uniform, being mainly around the periphery of the coverslip.

Analysis using FIB and SEM (Figure 86) showed slips 15 and 17 had only isolated areas of deposits. However, the deposits at the edge of slip 16 showed the desired deposition of nickel, filling all the pores of the alumina.
Figure 85. Nickel deposition onto coverslips: a. slip 15 (anodised to completion) showing the subtle interface (arrowed) between porous alumina of the left side and nickel deposition on the right side; b. slip 16 (anodised to cusp) showing non-uniform nickel deposition, with thicker depositions around the edges of the coverslip. The image shows the edge (i) with varnish immediately below it, nickel deposition (ii) and unplated or poorly plated porous alumina (iii); c. slip 17 (partially anodised) showing the edge of the coverslip (i), nickel deposits at the periphery (ii) and unplated or poorly plated central area (iii). Scale bars are 500 µm.
Figure 86. Nickel deposition onto porous alumina: a. Slip 15, showing isolated deposition above only a few pores that were correctly filled (Damage to the porous layer caused by a high FIB current can be seen by comparing the bare pores which have the appearance of being melted to the appearance of the pores protected under the nickel); b. Slip 16, showing correctly deposited nickel, near the edge of the coverslip. All pores in this area are filled with nickel, which has subsequently over-spilled the tops of the pores to form a continuous nickel electrode surface; c. Slip 17, again displaying poor, isolated nickel deposits. Scale bars are 1 µm.
EIS results for the coverslips are shown in Figure 87. The partially anodised and completely anodised slips showed the combined anodisation and deposition process produced a low impedance interface, comparable to the aluminium control. This was surprising, since only a few pores contained metal deposits. Since these small areas alone were unlikely to be responsible for the lowered impedance, it was speculated that the conductivity of the empty pores may also have been reduced.

The substrate anodised to cusp was broken by the EIS rig due to the thickness of varnish at the slip periphery. The rig was therefore modified by milling channels in the PTFE to provide clearance to the varnished edges.

The ‘stand-alone’ EIS testing using the Solartron and rig was time-consuming and only measured initial and final conditions. This gave no insight into the impedance changes during processing. It was therefore proposed that an Agilent 4294A could be used for real-time impedance measurements. This was investigated in the following experiment.
Figure 87. Solartron 1260A/1296 EIS data for deposition of nickel onto substrates that had been ‘partially anodised’ and fully anodised to ‘completion’. Data show that the processed substrates present an impedance similar to that of the aluminium control. Slip 9 data are included as a reference for unplated porous alumina showing the higher impedance. Area = 0.5 cm$^2$.

### 8.7 Anodisation Real-time Impedance Measurements

Real-time impedance measurement was devised using an Agilent 4294A Precision Impedance Analyzer. The purpose was to understand the impedance changes during anodisation and pore-widening (an unbiased etch) and to reduce the EIS measurement time: Solartron
measurements took approximately 7 mins per coverslip, plus ~5 mins to set up the coverslip in the rig. The 4294A is capable of performing fast sweeps (albeit to a minimum frequency of 40 Hz) and simultaneously forcing a d.c. bias (up to 40 V or 100 mA). It was proposed that the d.c. bias (voltage or current) could be used to anodise and thin the coverslips, with the thinning schemes programmed using the in-built iBasic scripting language. A review of the literature showed real-time impedance changes during anodisation had not previously been examined.

8.7.1 Methods

The iBasic programming language was used to develop a simple anodisation scheme with manual control. The program (Appendix C) consisted of two main subroutines, ‘Bias_dut’ for anodisation and ‘Measure_dut’ for EIS measurements. Data were automatically saved to a file. To provide feedback during processing, current and impedance (at $f = 110$ Hz) were plotted across the 4294A screen (Figure 88).

![Figure 88. Screen of the 4294A whilst running an iBasic anodisation program. The anodising current (upper trace) and impedance (lower trace) were plotted to provide feedback during processing.](image)

Thinning was by a linear voltage ramp followed by constant 5 V conditioning [344],[340]. Voltage ramps were used rather than stepped or ramped current since the former were thought
to be more readily scalable between the coverslip area and IC microelectrodes. Initial experiments on coverslips used linear voltage ramps. Exponential ramps were used for later coverslip experiments (and IC anodisation) as these better mimic the voltage decay during constant current thinning [340].

The output from the 4294A was used to bias the two electrodes in the anodising electrolyte (~2 cm² coverslip and platinum mesh counter electrode). The 4294A provides the capability for ‘compensation’ which eliminates the system components before saving impedance data. Compensation was performed by using two platinum electrodes, with the aluminium working electrode being temporarily replaced by a 1 cm² platinum plate electrode. The compensation was verified by testing with a 7.5 kΩ series resistor. This initial iBasic program used an anodisation voltage of 30 V with EIS readouts every 15 s. For a pore-widening experiment, a script was developed to anodise a substrate without electrochemical thinning, and then perform EIS readouts every 15 s without d.c. bias.

8.7.2 Results and Discussion
A completed 4294A anodisation script is included as Appendix C. During development of the program, impedance data were taken either during anodisation (i.e. testing whilst the device was biased with 30 V) or at open circuit potential (OCP). Expected impedance values were not observed whilst measuring during the 30 V bias. The reasons were not fully understood, but it was likely that the measurements were influenced by the active electrochemical (anodisation) processes at the working and counter electrodes. This would make an interesting topic for further work but was not investigated at this time.

The results of the unbiased pore-widening experiment are shown in Figure 89. The decreasing barrier impedance during pore widening was clearly seen (region c of the curve) and corroborates the literature of Table 11.

To validate the 4294A setup, the pore-widening data from the same coverslip (slip 21) were also tested on the Solartron to compare impedance measurements after anodisation and after the completed etching process where only the titanium layer remained (regions b and f

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61 The two sides of the Pt plate electrode being equivalent in area to the 2 cm² of single-sided coverslip.
respectively in Figure 89). Validity of the 4294A setup was confirmed by the results of Table 12.

![Impedance magnitude diagram](image)

Figure 89. Impedance magnitude, $|Z|$, at 300 Hz during pore-widening of a 2.0 cm$^2$ substrate in 4% phosphoric acid at 21°C: a. the initial aluminium substrate with $|Z| = 50 \Omega$; b. anodisation increases $|Z|$ to ~990 $\Omega$. The partial anodisation was stopped after 328 s leaving un-anodised aluminium and titanium below the porous film; c. the unbiased pore-widening etch results in a decrease in impedance as the barrier oxide and pore walls are thinned; d. the porous layer is eventually completely etched leaving a planar aluminium surface with impedance ~40 $\Omega$, similar to the initial surface; e. the remaining aluminium is thinned; f. the titanium layer is eventually reached and results a slightly higher impedance of ~53 $\Omega$, probably due to its slenderness of only ~40 nm.
Table 12. Comparison of Agilent 4294A and Solartron 1260A/1296 EIS data for impedance magnitude, $|Z|$ (normalised to unit area of 1 cm$^2$) and phase, $\Theta$. It is important to note that only the 4294A has system compensation. The Solartron data therefore includes solution series resistance and double layer capacitance (c.f. Randles cell, Section 8.4.2). The data from before anodisation are shown to be comparable, the additional 10 Ω·cm attributable to the system impedances of the EIS rig. The final electrode impedances are small and therefore the Solartron system impedance dominates the measurement. The phase data appear to be influenced by the system. However, the phase undergoes a rapid change ($d\Theta/df$) at $f < 100$ Hz and therefore such a discrepancy might be expected.

The results for impedance measurement during anodisation to the cusp and voltage-ramp thinning are shown in Figure 90. The initial data-point shows the impedance of the unmodified aluminium. The increasing impedance versus $f$ is due to the double layer at the solid-solution interface. Just prior to reaching the cusp it is interesting to note that the impedance ($40$ Hz $< f \leq 10^5$ Hz) starts to rise further before any change in anodising (d.c.) current can be seen. The cause of this is not understood, but perhaps could be an indication of barrier oxide deformation when forming immediately above the titanium layer.
Figure 90. Impedance magnitude, |Z|, versus frequency, f, and time, t, during anodisation to ‘cusp’ and voltage ramp thinning for a coverslip of area 2.0 cm$^2$. The same data are shown in 2D (a) and 3D (b): The impedance rapidly increases as the 30 V bias is first applied (i); The impedance remains constant during steady state anodisation (ii); just prior to the ‘cusp’ the impedance starts to rise slightly at all measured frequencies (iii); during the voltage ramp the impedance decreases for $f \leq 10^4$ Hz (iv).
8.8 Effects of Alloying Elements

In addition to the reduction of impedance by electrochemical thinning, it was discussed in Section 8.1.1 how the alloying elements present in CMOS metallisation might lower the barrier oxide impedance. This experiment was to investigate any such effects.

8.8.1 Methods

Coverslips were coated as previously (Teer Coatings, UK) except the aluminium was alloyed Al–1.0 wt%Si–0.5 wt%Cu to better represent the CMOS metallisation. Anodisation was performed in the standard phosphoric acid electrolyte, as above, with an additional coverslip anodised in 0.3 M oxalic acid electrolyte at 15°C for comparison.

8.8.2 Results and Discussion

Figure 91 shows the alloying elements result in reduction of barrier oxide impedance by a factor of 5.1 at $10^{-2}$ Hz. However, it can be seen that this factor is insufficient to make the substrate appear as conductive as an aluminium control.
Figure 91. EIS data showing the effect of the Si and Cu alloying elements. Slips 20 and 23 were anodised identically. At $f = 10^{-2}$ Hz, the impedance of the Al-Si-Cu substrate is a factor of 5.1 times lower.

Cross sections performed with the FIB showed the alloying elements caused disruption of the pore structure (Figure 92). The same disrupted pore structure was seen on alloy coverslips anodised at 60 V and on the substrate anodised in oxalic acid, suggesting it was the alloying elements causing the effect. Other anodisation studies of aluminium containing higher proportions of alloying elements than CMOS show similar disruption to the pore growth [222],[263],[264],[357],[358].
It was believed that the cross-linking of pores could be highly significant to the electrical model presented in Section 3.2.2. The model assumes that under the neuronal cell, isolated ‘columns’ of medium represented by \( r_p \) form conductors only to the corresponding location at the pore base. However, if adjacent pores are connected then this model increasingly breaks down as the breaches in the pore wall connect the area under the cell to those pores at the edge of the electrode. These may be connected to ground (via the bulk medium potential and bath electrode). As the proportion of hole in the pore wall increases, the alumina pores transition to being only a skeletal ‘sponge-like’ framework. The electrical model then effectively approaches that of a neuron being suspended \( \sim 1 \mu\text{m} \) above the true (titanium) electrode, with a large \( 1 \mu\text{m} \) cleft of medium below. It was decided it would be difficult to estimate the proportion of signal lost to this effect. If cross-linking of pores proved to be problematic, neuronal recordings would require the deposition of metal into the pores. The open electrode design would however still be useful for other biocompatible sensor applications where cell-substrate cleft size is not critical.

![SEM images of FIB sections showing the effect of CMOS alloying elements on pore structure](image1)

**Figure 92.** SEM images of FIB sections showing the effect of CMOS alloying elements on pore structure: a. pure aluminium (99.5\%) coverslip anodised at 30 V. The pore orientation varies slightly and so are not perpendicular to the FIB section surface. The length of uninterrupted pore wall therefore gives an indication of straightness; b. An Al-1\%Si-0.5\%Cu coverslip also anodised at 30 V. The pore walls are interrupted by cross-linking to adjacent pores. Scale bars are 500 nm.

### 8.9 Deposition Real-time Impedance Measurements

It was necessary to determine if the lower barrier oxide impedance of the Al-Si-Cu alloy would enhance electrodeposition. To improve understanding of the process, the anodisation real-time EIS capability was extended to electrodeposition of noble metal into the pores.
8.9.1 Methods

The 4294A analyser was programmed using iBasic to perform real-time EIS during deposition. However, the pulsed deposition scheme could not be generated by the 4294A and so the GPIB interface was used to remotely control the Agilent 33220A waveform generator. Additional problems arose due to an earth-loop (through the 4294A chassis) which could only be resolved by isolating the instruments from each other using a DPDT\textsuperscript{62} relay. The relay was driven (via a simple transistor driver circuit) from the TTL parallel port of the 4294A which was also controlled from iBasic. A hardware block diagram is shown in Figure 93 and the iBasic program is attached as Appendix D.

Comparison was made between pure aluminium and Al-Si-Cu coverslips. Anodisation was performed as previously, with each coverslip being anodised to completion ($I_{\text{steady}}/10$) followed by a voltage ramp to 5 V, then pore-widened for 39 minutes to further decrease the barrier oxide thickness. Nickel deposition was as previously, except pausing for EIS readouts every 15 s.

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\textsuperscript{62} Double Pole Double Throw
8.9.2 Results and Discussion

The real-time EIS during electrodeposition is shown in Figure 94. The rate of change of impedance appeared to become minimal after ~6500 s despite the coverslips still appearing transparent, suggesting little nickel had been deposited. The pure aluminium and Al-Si-Cu had similar $|Z|\cdot t$ characteristics with both coverslips still appearing transparent after deposition. Solartron EIS results (Figure 95) confirmed that the impedance of both samples had decreased to present a conductive electrode surface. However, comparison of the impedances at $f<40$ Hz indicated the alloying elements gave no benefit to the electrodeposition process, despite the previous experiment showing the impedance after anodisation and thinning was lower for the alloyed metallisation. It was therefore demonstrated that the electrical performance of the electrode can be improved with only a small deposit of metal, insufficient to reduce the transparency of the substrate. It was surmised that the poor deposition was most likely caused by inadequate or non-uniform thinning of the barrier oxide.

Figure 94. Real-time EIS during nickel electrodeposition ($f = 110$ Hz). The dip at 3649 s is due to the process being paused for a Solartron EIS measurement (Slip 25, pure Al, anodised to completion, deposition at 35°C). Impedance data were collected for $40 \leq f \leq 1 \times 10^6$ Hz but for clarity only a single frequency is presented here.
Figure 95. Solartron EIS comparing deposition on pure aluminium versus Al-Si-Cu coverslips. At low frequencies, the impedance of both coverslips has reduced during deposition (from an impedance similar to the porous alumina control) to below that of the aluminium control. However, the results show little difference between the pure aluminium and Al-Si-Cu.

### 8.10 Reverse Bias Barrier Oxide Removal

The previous experiments indicated that an improved barrier oxide thinning process was required. As summarised in Table 11, success has been reported using a reverse bias after anodisation. In [343] an Al/Ti/Si substrate was anodised in sulphuric acid, followed by a reverse bias to generate hydrogen gas. It was concluded the process is driven by the
generation of H$^+$ ions at the pore bases causing a localised decrease of pH and so etching the oxide. In [347] it was found that the process required careful control to avoid rapid gas generation from rupturing the porous alumina and lifting it from the substrate. It was found a suitable visual control was to increase the magnitude of the reverse bias until gas formation could be seen, then to slightly reduce the bias magnitude until no further gas generation could be observed. In [346] a similar process was performed but in a potassium chloride electrolyte with a bias of $-2.25$ V for several minutes.

Due to the requirement for a simple low-cost process, it was decided to adapt the above processes to the phosphoric acid electrolyte. It was postulated that the process performed in sulphuric acid could also be performed in phosphoric acid since hydrogen gas should be generated in a similar fashion under reverse bias.

### 8.10.1 Methods

To determine a suitable bias, the visual method from [347] described above was used with an anodised coverslip. This was then used to develop an iBasic program to apply short (5 s or 10 s) reverse bias pulses. As previously, the edges of the coverslips had been sealed with varnish to avoid edge effects (e.g. high electric fields).

To characterise the effect of bias magnitude, a set of coverslips were anodised and thinned to $V = 5$ V, with different reverse biases then applied to each coverslip ($-1.44$ V, $-1.96$ V and $-2.25$ V). All coverslips were then plated with nickel using the same method as previously.

### 8.10.2 Results

The preliminary coverslip tests showed lots of gas generation on the surface of the substrate at $-2.50$ V. Reducing the bias to $-2.25$ V resulted in the desired slow gas evolution. This was noted to be the same potential used in [346].

A gradual change in $|Z|$ was expected as a result of barrier oxide dissolution. Surprisingly however, initial tests with an iBasic program showed a rapid collapse in $|Z|$ within a short

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$^{63}$ These potentials were derived from a preliminary experiment where the voltage of pulses were increased exponentially where $V = -x^2$, where $x$ is increased from 1.0 to 2.0 in steps of 0.2.
period (5 s) of a reverse bias being applied (Figure 96). The lowered impedance was maintained for a considerable time after removal of the bias suggesting this was not a transient response or an artefact in the impedance measurements. Varnishing the coverslip edges should have prevented edge effects from affecting the results.

![Graph](image-url)

Figure 96. The effect of reverse bias on impedance. The upper graph shows $V$-t and $I$-t during anodisation, with the lower graph showing $|Z|$-t at $f = 110$ Hz (other frequencies $40 \leq f \leq 10^6$ Hz showed a similar response). Both graphs share the same time axis. After completing the V-ramp thinning at $t = 2097$ s, a single $\sim 4.0$ V bias was applied for 5 s resulting in a fall in $|Z|_{110Hz}$ from 1.0 kΩ to 200 Ω (slip 30).
The above coverslips (biases of $-2.25 \, \text{V}$, $-2.5 \, \text{V}$ and $-4.0 \, \text{V}$) with the additional set of $-1.44 \, \text{V}$, $-1.96 \, \text{V}$ and $-2.25 \, \text{V}$) were plated with nickel, as previously. SEM analysis of FIB sections showed three features:

i. Nickel deposition was much improved (thicker) compared to previous experiments (Figure 97).

ii. Substrates processed with a higher reverse bias seemed to have more damage to the porous alumina layer (Figure 98), although this relationship could not be fully asserted.

iii. Pore walls within the nickel were observed under the SEM to be extremely thin (Figure 98).

Figure 97. Coverslip plated after reverse bias ($-1.96 \, \text{V}$, 10 s, slip 32). The left side is unprocessed aluminium (control). The central area is porous alumina. The right third shows a reflective (thick) coating of nickel. (The coverslip length from left to right is 32 mm.)

Figure 98. SEM image of FIB section through a nickel-coated region of slip 35. The white arrow indicates the porous layer, with what is believed to be the pore walls appearing high contrast but very thin and/or broken. The black arrow indicates nickel deposition above the porous alumina, with the metal grain structure clearly visible using the in-lens detector. The height of the porous layer varies due to damage during anodisation and thinning (as confirmed by additional SEM analysis of the AAO control segment of the coverslip). Scale bar is 500 nm.
8.10.3 Discussion

The mechanism under investigation was expected to be chemical dissolution due to localised acidification at the pore bases caused by \( \text{H}^+ \) ion evolution. However, the rapid (< 5 s) decrease in impedance observed was considered too fast to be chemical dissolution. It is postulated that the response may have been due to dielectric breakdown of the barrier oxide \([359]\). The barrier oxide thickness is known to be approximately 1.1 nm$\cdot$V$^{-1}$ \([218]\) and so the mean oxide thickness is ~5.5 nm after the anodisation ramp to 5 V. The application of, say, a 1.44 V reverse bias therefore represents a field of 261 MV$\cdot$m$^{-1}$. This magnitude of field strength is known to cause dielectric breakdown in aluminium oxides \([360]\).

This reverse bias mechanism therefore appears to be a useful technique for reducing the impedance of the barrier oxide in a porous alumina electrode, resulting in improved electrical characteristics beyond those achievable using only anodising voltage-ramps and pore-widening. The reverse bias might also be used as a pre-conditioning process for a subsequent electrodeposition step by lowering the impedance at the pore bases but such a benefit remains unproven.

However, further characterisation of the mechanism is required to understand the damage caused to the porous film. It is also acknowledged that the above tests have not yet led to a full understanding of the mechanism and its controlling factors. Breakdown voltage could perhaps be better characterised by applying either a fixed potential or a more finely controlled pulse, each applied to a fresh coverslip. Unfortunately this was too costly for this present work as it requires a large number of anodised coverslips.

The above experiments demonstrated that nickel deposited into the porous alumina reduced the electrode impedance, but the alumina pore walls were found to be extremely thin.

8.11 Modulation of Pore Wall Thickness

From the preceding experiment it was found that the walls of the pores were very thin. It was proposed that the walls needed to be thickened for two reasons: firstly, it was shown in Chapter 6 that porosity manipulation is required to achieve the desired cell adhesion,
especially at the low anodising voltages which are most easily implemented in CMOS. Secondly, as described in Section 3.2.4, retaining the porous structure maintains the ability to provide a flexible foundation for a range of electrode designs. For example, to produce a dendritic metal electrode morphology it would be necessary to partially fill the pores with metal then partially etch back the porous alumina walls to expose the metal pillars.

Lower porosities can also be achieved by anodising at higher voltages, but this increases the risk of incompatibility or design difficulties with CMOS. Alternatively, lower porosities can be achieved using oxalic or sulphuric electrolytes, but anodising at suitable voltages requires the temperature to be lower than room temperature to avoid burning and high current density [361]. This would require cooling apparatus that does not fit well with the thesis objective of developing a low-cost manufacturing process.

As discussed in [362], the addition of polyethylene glycol (PEG) to an anodising electrolyte reduces the acid’s pore-widening action and so reduces the porosity of the film (i.e. thickens the pore walls). This experiment therefore aimed to reproduce this effect to retain thicker pore walls after anodisation.

### 8.11.1 Methods

Two sets of coverslips were anodised as for the preceding experiments (30 V anodisation to cusp; voltage ramp to 5 V). The first set was designed to reproduce the work of [362] and so was anodised using similar conditions (2% w/v phosphoric acid, 15°C, with varying percentages of PEG-400 (30% w/v, 50%, 80%). The second set was processed at room temperature to meet the constraint of low-cost CMOS processing (21°C, 40% w/v PEG-400) and with varying concentrations of phosphoric acid (0.5% w/v, 1.0%, 2.0%, 4%). To maintain the accuracy of EIS measurements, separate 4294A compensation files were generated for each PEG electrolyte due to the different conductivities (See Section 8.7.1). Surface porosity was measured using ImageJ.

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64 The CMOS ICs were not yet available during these experiments and therefore high voltage anodisation using the ICs could not be tested.
8.11.2 Results and Discussion

The effect observed in [362] was reproduced on the Al-Si-Cu coverslips anodised in 2% w/v phosphoric acid at 15°C. The desired porosity manipulation was then successfully transferred to a room temperature process by using the reduced phosphoric acid concentrations (with 40% PEG): reducing the phosphoric acid concentration from 4% to 0.5% resulted in halving the surface porosity (Figure 99).

However, it seemed there was an upper limit to the usefulness of this technique since coverslips anodised with low (0.5%) phosphoric acid concentration or >50% PEG resulted in poor quality films exhibiting reduced porous alumina thickness. This was visible to the naked eye as pin-holes through the substrate and was confirmed by FIB sections under the SEM.

![SEM images showing the effect of surface porosity manipulation using 40% PEG-400 at 21°C with varying electrolyte concentrations (w/v): a. 4% H₃PO₄; b. 2% H₃PO₄; c. 1% H₃PO₄; d. 0.5% H₃PO₄. Anodisation was at 30 V with barrier oxide thinning by voltage ramp to 5 V. Porosities, measured using ImageJ software, are 68%, 48%, 36% and 32% respectively. Scale bars are 500 nm.](image)

Therefore, by using 40% w/v PEG-400 with only 1% w/v phosphoric acid instead of the standard 4% w/v electrolyte, a room temperature method is available to reduce surface
porosity by up to ∼47% (Figure 100). This technique can be used as an alternative to, or in combination with, higher voltage anodisation, which also decreases porosity.

Figure 100. Manipulation of pore wall thickness: a. After anodising with the standard 4% phosphoric acid electrolyte the pore walls are barely discernable (Anodised at 30 V, V-ramp to 5 V, 21°C. Nickel deposition. Slip 35); b. After anodising in 1% phosphoric acid and 40% PEG the pore walls are thicker (Anodised at 30 V, V-ramp to 5 V, 21°C. Nickel deposition. Slip 62). The image shows a high contrast nickel ‘mound’ (i) with unfilled pores (ii) above. The pore walls within the nickel appear as low contrast areas (iii). This change of contrast is an artefact of the electron scanning and secondary electron detection, and was checked by carefully tracing pore walls across the nickel-vacuum boundary. Scale bars are 400 nm.

8.12 Improved Platinum Electrodeposition

Previous experiments had demonstrated that a framework of porous alumina could be anodised and electrochemically thinned producing a sufficiently low impedance to enable electrodeposition of nickel into pores and where the porosity had been lowered to maintain the porous structure. Attention therefore returned to depositing a noble metal rather than nickel. The objective of this experiment was therefore to apply platinum deposition to the cumulative improvements in the barrier thinning process, the real-time impedance measurement tools, reverse bias impedance reduction and porosity manipulation.

Initial trials of platinum deposition gave poor results, suggesting that differences between the nickel and platinum deposition processes were responsible. Coverslips in P-salt were
observed to generate gas bubbles at the working electrode whereas similarly anodised substrates in the nickel Watts Bath were observed to plate well without producing visible quantities of gas. A series of experiments was therefore proposed to improve the platinum deposition. Several factors were considered:

i. **pH of P-salt bath**: The bath had been chosen for its neutral pH due to the corrosion problems seen with the CPA bath at room temperature. However, the buffer was shown in Section 8.5.2 to cause dissolution of aluminium and alumina at typical P-salt bath operating temperatures of 89–95°C [330]. It was considered whether these problems were being compounded by the relationship between current efficiency and pH. In [330] it is explained how a high (>50%) current efficiency is only achievable for P-salt at temperatures in excess of ~80°C. There is a sudden fall in efficiency below ~60°C to ~10% at 20–50°C. It is further explained that that there is an almost linear relationship between pH and current efficiency (a pH change of ~4 for an efficiency change of about 40%). Therefore, it is possible that the pH at the pore base was much lower than the pH 7 of the bulk electrolyte.

ii. **Gas**: It was considered whether the visible gas was a cause of the problems or a symptom. For example, gas formed at the base of the pores could have been inhibiting deposition. Conversely, a lack of deposition due to some other cause could result in hydrogen being produced as the dominant chemical process.

iii. **P-salt temperature**: as explained above, high bath temperature caused dissolution but low temperature resulted in poor current efficiency. This might leave a ‘process window’ but it was not clear how wide this window was or whether it existed at all.

iv. **Bath type**: Initial plating tests showed a standard CPA bath caused rapid dissolution of the films. It was considered whether the pH of the CPA could be adjusted to ~7 (as performed in [363] using a Na₂HPO₄ buffer) or whether alternative baths could be used such as Dinitrosulfato Platinum (‘DNS’) operated at its 30°C optimum [364].

v. **Nucleation**: the poor coverage of platinum could be due to a nucleation problem and would explain the gas evolution as a symptom. Conversely, it is known that too large a potential results in an ‘over-potential’ that will tend to produce hydrogen rather than metal deposition [335], [342]. In [339], gold chloride (H₂AuCl₄·3H₂O) was deposited for 6 s to provide nucleation sites at pore bases prior to subsequent deposition into porous alumina.

vi. **Waveform**: The +70 mA/−3 V pulsed electrodeposition (PED) scheme used in previous experiments was adopted from [334], [335], [337] and [365] wherein they
state that the large current density and low duty cycle are intended to ensure good plating efficiency by preventing gas evolution caused by depletion of metal ions at the pore bases and by achieving good nucleation by overcoming the potential barrier presented by any remaining insulating barrier oxide. However, other electrodeposition schemes have also been used to deposit nanowires into porous alumina templates: d.c. was used by [215], [333], [339], [341], [345], [349] [353], [366] and [367]; pulsed d.c. by [349] and [366]; a square wave with d.c. offset was used by [334]; a.c. used by [342], [348], [351], [354], [350] and [365]; a pulsed +/- current scheme was used by [344] and [351].

vii. Barrier oxide thinning: nickel deposition had been achieved using the electrochemical voltage ramp thinning process. It was possible that the platinum bath could be more sensitive to the barrier impedance than the nickel due to differences in bath chemistry.

Experiments were therefore performed to understand better the above factors.

8.12.1 Methods

The objective of the experiment was to increase plating thickness and uniformity without damaging the porous layer. The effects of each variable were tested independently rather than attempting to study all interactions via a single multi-factorial experiment. For a nucleation tests, gold chloride was used prior to P-salt deposition (1.0 g.l⁻¹ HAuCl₄.3H₂O, 7.0 g.l⁻¹ H₂SO₄, 15 s, 50 mA·cm⁻²) and non-standard pH 4, 5 and 7 CPA baths were tested (8.9% w/v Na₂HPO₄, 4% w/v CPA, pH adjusted with H₃PO₄ or NaOH). To analyse further the effect of P-salt temperature, substrates were plated for 1 hour at 35°C, 45°C, 55°C and 65°C. To analyse effect of plating time, further coverslips were plated for extended periods of 2 and 3 hours. An attempt to overcome any potential barrier was tested by increasing the pulsed electrodeposition potential to 9.9 V (≡6.25 V at the electrode). To evaluate duty cycle, the waveform period of the +70 mA·cm⁻²/-3 V scheme was changed from 500 ms (1.6% duty) to 100 ms (8%), 53 ms (15%), 32 ms (25%) and 16 ms (50%). To avoid excess gassing and potential damage to the AAO, depositions were started at a duty no greater than 8% for the first 5 mins.

For effects of barrier oxide impedance, substrates were partially anodised, anodised to cusp and anodised to completion. To improve SEM image detail (e.g. oxide thinning at pore bases,
pore walls), the anodising potential was increased to 60 V. As this exceeded the 40 V of the 4294A d.c. output, a Keithley 2400 SourceMeter was used, controlled from the 4294A iBasic program via GPIB (Figure 93).

To assist in the understanding of the P-salt electrochemistry, a µAutolab Type II or PGSTAT2 (Metrohm Autolab B.V., Netherlands) potentiostat (‘p-stat’) with GPES software v.4.9 was used to perform cyclic-voltammetry (CV). The working electrode was porous alumina coverslip (anodised at 60 V and barrier oxide thinned as for previous experiments) with platinum counter electrode and a saturated calomel electrode (SCE) as a reference. The scan rate was 0.02 V·s⁻¹, cycling between −0.5 V and −1.2 V.

Fresh P-salt baths were regularly prepared as they were found to be unstable, resulting in platinum precipitates after only a few days or a few weeks.

8.12.2 Results and Discussion

The CV experiments using P-salt at 22°C showed the OCP was approximately −0.2 V. The loop formed between the negative and positive scan directions was indicative of electrodeposition. Scans indicated that deposition occurred at potentials of magnitude as small as −1.0 V. Extended cycling up to 1½ hours resulted in a visible deposition but still left the coverslip transparent, suggesting that only a thin deposition was present. No difference was seen between coverslips partially anodised or anodised to cusp or to completion. For a CPA bath of pH 4, the OCP was ~0.165 V but extended CV cycling resulted in no visible plating.

All CPA bath experiments, using both constant current and +70 mA/−3 V PED schemes, resulted in either no or poor depositions. The most successful CPA depositions resulted in visible changes to the films, although the coverslip remained transparent and had pin-holes. SEM analysis showed only isolated regions of platinum deposition which seems to have nucleated at only single locations, possibly relating only to defects in the porous alumina film. The alumina was also observed to have lifted from the substrate (Figure 101).
Figure 101. Electrodeposition using a CPA pH 4 bath (slip 77): a. low magnification SEM image showing only isolated areas of deposition. The bare areas were responsible for the coverslip maintaining its transparency. The arrows indicate lifted films which are probably the cause of the pin-holes. (Scale bar is 500 µm); b. At higher magnification an FIB section shows the isolated areas of deposition had nucleated at a single point on the substrate, possibly relating to a defect in the porous alumina. Lifting of the alumina is also visible on the left side of the image. (Scale bar is 1 µm).

Experiments to evaluate plating duration and duty cycle showed significant effects (the active plating time being regulated by both of these parameters). Figure 102 shows the effect of duty cycle. This illustrated that the low duty cycle (1.6%) used by [337] and others was not optimised for the P-salt conditions used here and that higher duty cycles gave better results (i.e. thicker platinum without porous film damage).
The results of the experiment to re-evaluate the effects of P-salt bath temperature are shown in Figure 103. This was performed at a 50% duty cycle for 55 mins (after the initial 5 mins at 8% duty) with the premise that at higher temperatures there was a race between fast plating to quickly cover the alumina against the dissolution of the film (c.f. Section 8.5.2).
Other factors had less significant effect on plating or caused problems, as assessed using visual inspection, optical microscopy and SEM / FIB: Constant current resulted in either copious gas evolution (P-salt, 15 mA·cm\(^{-2}\)) or non-uniform deposits (CPA pH 7, 15 mA·cm\(^{-2}\)). Raising the potential of the PED scheme (with resulting current varying between 50 mA·cm\(^{-2}\) and 90 mA·cm\(^{-2}\)) resulted in an opaque (thick) platinum film but with lots of pin-holes signifying damaged alumina. There was no evidence that the gold chloride pre-deposition step improved platinum P-salt deposition.

Having achieved deposition of platinum into the porous alumina, the +70 mA/−3 V PED scheme was revisited to determine if it could be simplified. The circuit was replaced by a simple positive pulse (100 ms period, 10–50% duty) generated directly by the 33220A.
waveform generator. This produced results indistinguishable from those achieved with the +70 mA/−3 V circuit. It is therefore likely that, for our thin 1 μm substrates, the simplified PED scheme is acceptable due to the lower aspect ratio of the porous alumina compared to the porous films of up to 50 μm used by others.

8.13 Conclusions
The impedance of porous alumina without thinning of the barrier oxide was shown to be sub-optimal for use as an electrode (2.4 x 10^7 Ω·cm at 10^-2 Hz). When anodised at high voltage, deformation of the barrier oxide could be observed using the SEM but there was no evidence of the physical defects in the oxide observed in [233]. A moderate reduction of impedance was achieved by barrier oxide thinning alone. Improved conductance was achieved by deposition of platinum (or nickel) into the porous alumina (3.8 x 10^3 Ω·cm at 10^-2 Hz). Impedances better than the aluminium control (1.1 x 10^5 Ω·cm at 10^-2 Hz) were readily achievable despite poor platinum coverage. Deposits not visible on the FE-SEM were sufficient to cause these improvements in the electrical characteristics. It is plausible that this is due to a reduction of the oxide electronic band gap caused by diffusion of the metal ions into the oxide as postulated by [234]. This technique can therefore be used to improve the conductance of the ‘plain’ porous alumina electrode design.

A novel method for real-time impedance measurement during anodisation was developed and validated. For the first time, the barrier oxide impedance was measured during a pore-widening etch and during electrochemical (linear voltage ramp) thinning. The technique was extended to measure real-time impedance during electrodeposition. These results were validated and supplemented by stand-alone EIS data, incorporating a cell specially developed to interface the coverslips to the impedance analyser.

Alloying elements present in the CMOS metallisation (1% Si, 0.5% Cu) were shown to affect the physical and electrical properties of the porous alumina. FIB sections showed a disrupted pore structure with cross-linking of adjacent pores. The alloying elements decreased the magnitude of the barrier impedance by a factor of 5.1 and the phase indicated it was more resistive than the barrier oxide formed from pure aluminium. The cross-linking is highly significant to the proposed model for neuronal recordings. If the cross-linking proved
problematic, electrodes for neuronal recordings would require deposition of metal into the pores.

Attempts to thin the barrier oxide by applying a reverse bias to generate hydrogen gas and physically remove the barrier oxide did not have the anticipated outcome. Instead, it was found that the reverse bias method represents a potentially novel technique that was shown to reduce the thinned barrier oxide impedance by a further factor of 5. It was hypothesised that the mechanism is likely to be electrical breakdown of the barrier oxide. However, there were additional indications that the process might also damage the porous film and therefore this method remains an area requiring further research.

To prevent the porosity of the alumina film from becoming too high and negatively impacting cell adhesion, it is necessary to control the porosity during electrode fabrication. Thinning of the pore walls caused by, for example, low voltage anodisations or etching by electrodeposition electrolytes, can also result in an undesirably high porosity. A method using an anodising electrolyte incorporating PEG was adapted for the first time to operate at room temperature. It was demonstrated that this successfully increases the alumina pore wall thickness.

Platinum deposition was neither successful using a standard chloroplatinic acid bath (pH 0) nor a buffered CPA bath (pH 4–7). Using a P-salt bath (with neutral pH as prepared) gave modest improvements, but demonstrated difficulties with damage to the porous alumina and slow plating, requiring 1 hour at a 50% duty cycle to achieve a film thickness of only ~450 nm. At this point it was unclear whether scaling from the coverslips (1 cm²) to the CMOS pads (700 µm²) would improve uniformity of the plating or introduce additional problems.
Key Points

- A novel real-time impedance technique has been developed to give insight into anodisation and electrodeposition processes.
- Alloying elements in CMOS metallisation were shown to affect alumina pore structure and electrical characteristics.
- A reverse bias across the porous alumina film resulted in a further 5 x reduction in impedance. This was believed to be caused by electrical breakdown of the barrier oxide.
- Platinum deposition into porous alumina was demonstrated. Non-standard plating baths were required and careful control of parameters was necessary to avoid damaging the aluminium and porous alumina substrate.


9 CMOS Electrode Processing

9.1 Introduction

Several biocompatible CMOS electrode designs were proposed in Chapter 3: the porous alumina could be used directly as the electrode or the pores could be filled with a noble metal to form an electrochemically stable and biocompatible planar surface. Chapter 8 investigated the electrical characteristics of a porous alumina electrode, showed how they can be optimised and demonstrated electrodeposition with platinum. By combining these methods with the design and assembly work of Chapter 7, the post-processing of the CMOS ICs (Figure 71, Figure 72) could now be explored.

Two forms of electrode were to be investigated: firstly, the plain porous alumina as a simple general-purpose biocompatible electrode and, secondly, a planar noble metal electrode where the pores are filled with a noble metal. The following presents this aspect of the research in a sequence of experimental sections. Firstly, initial experiments were performed to demonstrate that the work using coverslips could be transferred to the CMOS ICs. Problems discovered in the first section are addressed in subsequent sections.

9.2 Preliminary Processing Experiments

The object of these experiments was to transfer the results from the coverslips to the CMOS ICs. Generally, it was expected that the voltages would remain the same but currents would scale with electrode area. Aspects of the processing to be reproduced on the ICs were anodisation, barrier oxide thinning and electrodeposition of a noble metal.

9.2.1 Materials and Methods

Experiments were performed using the CMOS ICs with an array of 48 planar microelectrodes. The unprocessed CMOS pads comprised 40 nm of titanium followed by 960 nm of Al–1.0 wt%Si–0.5 wt%Cu alloy. Coverslips with Al-Si-Cu were used to scale the electrodeposition conditions.
Unless otherwise stated, anodisation was performed using a 0.4 M (4 % w/v) phosphoric acid electrolyte at 21°C. The apparatus used for anodisation and electrodeposition was principally the same as that for the preceding work in Chapter 8. The Keithley 2400 SourceMeter was used instead of the 4294A d.c. bias since the 2400 SourceMeter was able to supply potentials in excess of 40 V and could measure the small (nA) IC anodising currents. A printed circuit board (pcb) with zero insertion force (ZIF) socket was used to connect all IC pins together so that all pads were anodised in parallel.

The 4294A was again used to perform real-time EIS during anodisation and electrodeposition, but ambient noise reduced the accuracy of readouts when the electrodes where anodised (to a high impedance) but had not yet had their barrier oxides thinned. Turning off the hood extractor fan was found to reduce the electrical noise. Stand-alone EIS was performed using the Solartron 1260A/1296 with a 1 cm² platinum plate placed in the culture chamber to form the counter electrode.

For deposition onto IC microelectrodes, the same pcb (with ZIF socket) was used as for anodisation, so all the pads would be plated in parallel. The P-salt bath was used with the 35°C operating temperature maintained using the ‘RCT Basic’ hotplate. Due to the limited volume of the culture chamber, the temperature probe was placed in the culture chamber of an adjacent ‘dummy’ IC (taken from previous Bath / King’s College London work) which was filled with water (Figure 104).

A pulsed deposition scheme was used based on the results of Section 8.12, starting with 5 minutes at 8% duty (8 ms) followed by the remaining time (up to 1 hour) at up to 50% duty. The counter electrode potential was set by scaling from the coverslips. This was achieved by plating a coverslip with the pulsed scheme whilst a reference electrode (SCE) in the plating bath was connected to an oscilloscope. This allowed the potential of the bath (and hence the potential across the working electrode, i.e. the porous alumina) to be determined.65

65 The potential across a counter electrode typically drifts, which is why a reference electrode is often required in electrochemistry.
Figure 104. IC deposition temperature control: the IC being plated is mounted in a ZIF socket (i) on a pcb. The platinum counter electrode (ii) is suspended in the culture chamber. The built-in hotplate temperature sensor could not be used with this configuration due to the remoteness of the culture chamber to the hotplate. A second ‘dummy’ IC (iii) is therefore configured in a similar manner to the IC being plated but is used solely as a temperature reference. The culture chamber height of the dummy IC has been extended using an additional glass ring so that the minimum length of the temperature probe (iv) is submerged in the water.

The SEM / FIB dual-beam 1540XB system was used for all analysis. The FIB enabled precision cross sectioning of processed IC pads (generally using 2 nA current and energy of 30 kV). Due to the close working proximity of the SEM column, it was necessary to disassemble the ICs prior to imaging. ICs assembled using the PEG-mould process were used. They were prepared for the SEM by removing the culture chamber and the elastomer using a modelling knife. This was a destructive process since the bondwires are pulled away with the elastomer. The ICs were therefore used sparingly.

9.2.2 Results and Discussion

9.2.2.1 Anodisation

The three principal anodisation schemes were successfully reproduced on the ICs (Figure 105).
Figure 105. IC anodisation demonstrating the three barrier thinning schemes developed using coverslips: a. ‘cusp’ – the exponential voltage ramp is triggered by a fall (i) of current from the steady state; b. ‘completion’ – the exponential voltage ramp is triggered (ii) when the anodisation current has fallen to 10% of its steady state; c. ‘partial’ – the exponential voltage ramp is initiated after a fixed period (iii), calculated as 50–70% of the duration to the cusp. Drop off in currents during initial ramping (iv) indicate the barrier oxide temporarily falling out of the anodising breakdown state. The desired approximation to a linear current ramp can be seen in the latter stages of the voltage ramp (v). The arrows indicate the corresponding y-axis. Data are for the array of 48 electrodes (in parallel) with combined area of $3.4 \times 10^{-8}$ m$^2$. 
FIB and SEM imaging confirmed correct anodisation of the electrodes. Figure 106 shows an anodised pad. The detail at the edge of the pad shows anodisation undercutting the passivation. This was believed to be particularly beneficial since the remaining aluminium track is protected from corrosive media by a buffer zone of porous alumina.\footnote{This protective zone would not be present in electrode designs that do not incorporate the porous alumina buffer. For example, the titanium electrode of Figure 29d would leave aluminium track exposed at the pad edge.}

It was a concern that, due to the Pilling-Bedworth ratio (for metal oxide to metal) of 1.28–1.70 for aluminium [368], the alumina would exert a force on the passivation. It had been unclear whether the stress within the alumina would be contained chiefly within each individual pore region (which might be expected, as this is the very mechanism by which pores form) or would act on the passivation and cause its failure. However, there was no evidence of passivation cracks.\footnote{Whilst not seen here, such stresses are frequently the cause of reliability failures in high volume applications. Future work is required to perform semiconductor reliability tests to confirm passivation cracks do not appear with time.}

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9.2.2.2 Deposition

Using the three electrode cell with the SCE connected to the plating bath, it was possible to scale the coverslip plating voltages for ICs. With 0 V d.c. applied to the system, the oscilloscope showed an offset of ~250 mV. This corresponded with the known potential of 241 mA for an SCE at ~30°C [369]. The potentials measured during the plating of the coverslips are shown in Table 13. The difference between the 33220A set voltage, $V_{SET}$, and measured system potential, $V_{SYS}$, is due to the internal impedance of the generator (i.e. limited current capability). The potential across the working electrode is the difference between $V_{SYS}$ and $V_{SCE}$, and can be seen to be minimal for set potentials $V_{SET} \leq 1.0$ V. For larger set potentials the counter electrode potential became significant. It could therefore be determined that plating of an IC array with the equivalent potential of $V_{SET} = 4.5$ V would require a bath
potential of ~1.75 V. Repeating the procedure during IC plating showed no differences between the set potential on the 33220A and bath potential measured by an SCE. Therefore deposition onto ICs required the 33220A to be set to the same voltage as the desired $V_{SCE}$.

![Oscilloscope output](image)

**Figure 107.** Oscilloscope output illustrating the measurement of $V_{SYS}$. The upper trace shows an 8 ms pulse with $V_{SYS} \approx 3.0$ V (which is the potential measured across the system). The lower green trace is the measured potential at the SCE and shows the nominal $V_{SCE}$ (whilst $V_{SYS} = 0$ V) is approximately −1.0 V. This is a potential across the porous alumina. During the pulse, the potential at the SCE increases to $+1.5 \pm 0.25$ V. The lower blue trace is the current (the potential across a $1 \Omega$ resistor) with 20 mV/div $\equiv 20$ mA/div.
Pulse amplitude set on Agilent 33220A waveform generator\textsuperscript{68}, \( V_{SET} \) (V) | Measured system potential across counter electrode and porous alumina working electrode, \( V_{SYS} \) (V) | Bath potential (across working electrode) measured by SCE\textsuperscript{69}, \( V_{SCE} \) (V) |
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Table 13. Determination of plating bath potentials using an SCE and oscilloscope (Slip 101, P-salt, 35°C). Resolution of \( V_{SCE} \) is ±0.25 V.

Real-time EIS showed the impedance of the electrode arrays to generally reduce during platinum electrodeposition (Figure 108). However, the response was inconsistent: the impedance of some arrays increased (e.g. IC11), some decreased steadily (e.g. IC13, IC19) and many had rapid drops in impedance (e.g. IC12, IC15).

\textsuperscript{68} The limited current capabilities of the Agilent 33220A result in the voltage present at the output being different to that set, as seen at set potentials >3.0 V. The 33220A allows adjustment for a 50 \( \Omega \) load or high-impedance, but not for variable loads as used here.

\textsuperscript{69} Values shown exclude the fixed 242 mV offset across the SCE electrode.
Visual inspection showed many ICs had electrodes that had darkened, but also frequently had deposits to one side (Figure 109). This had the characteristics of a plume that had been ejected from the electrode and had subsequently settled beside it. SEM / FIB analysis showed that the porous alumina had been etched at its base, with increasing proportions of the alumina being removed in an action working upwards toward the electrode surface (Figure 110). This provided further evidence to show the plume was debris from the pad and was probably the remains of porous alumina that had been ejected as the film had lifted. It was speculated that the rapid decreases in impedance were due to sudden failure of the pads. The lowered impedance was then due to the titanium layer acting as a planar electrode. This was supported by SEM evidence where platinum had subsequently started to deposit on the exposed titanium layer.
Figure 109. IC array after P-Salt electrodeposition. All plated pads have darkened, with many displaying what appears to be a plume of material from the electrode that has subsequently settled on the passivation surface. As a control, a quarter of the array (pins 1–12, marked in this image with a red border) was unbiased during deposition (IC5. Pad diameter is 30 µm).
Figure 110. SEM images showing results of P-Salt deposition onto IC arrays: a. FIB section showing no platinum in the pores, but voiding at the pore bases. Some platinum can be seen at the bottom right of the image which is incorrectly deposited on the alumina surface (IC3, pin 38); b. A pad with most of the porous film etched from its base and showing the remainder of the porous layer to be lifting out of the pad opening (IC5, pin 29); c. A porous alumina film lifting from its pad opening (top left side) with debris visible on the passivation in the top left corner of the image (IC4, pin 13). All scales bars are 2 μm.
Hydrogen gas evolution was inferred by the lack of platinum, the dissolution of the pore bases and the lifting of the porous film, and that P-salt experiments using coverslips had also shown loss of porous alumina film as well as copious gas evolution observed by eye. Further experiments were therefore performed in an attempt to eliminate these issues. The plating potential was decreased to 2.0 V to reduce any over-potential and gas evolution. In another test, the potential was increased to 4.5 V in case the potential across the alumina barrier oxide was preventing platinum nucleation. It was also considered whether depletion of dinitrodiammine-platinum ions at the base of the pores could have been causing gas evolution. To mitigate this problem, bath agitation was introduced by using a syringe and continuous reciprocation of the plunger to flow solution across the array. These attempts were generally unsuccessful, except for a moderate improvement attributed to the agitation (resulting in a reduced number of lifted films).

A further review of coverslip plating experiments showed that deposition at constant pulsed voltage resulted in an increasing current during plating over the typical period of up to 1 hour. Additionally, it was already known that the electrode impedance decreased with plating time (Figure 108). It was therefore considered whether this was a positive feedback condition and causing excessive current to flow through the electrodes.

### 9.3 Pad Damage Mitigation Experiments

To investigate the possibility that excessive current was damaging the electrodes, the pulsed electrodeposition scheme was changed from voltage-controlled to current-controlled. Additionally, a gold chloride bath was used as an attempt to identify whether the problems with porous alumina dissolution and lifting were specific to the platinum bath.

#### 9.3.1 Methods

The experimental conditions were similar to Section 9.2 above, including agitation of the bath using the syringe technique. The deposition current was limited using the circuit of Figure 111. The 22 kΩ potentiometer allowed the current to be precisely adjusted and the 10 kΩ resistor provided a shunt across which an oscilloscope was used to adjust and monitor
the current. The same current density was maintained between coverslips and IC pads (20 mA·cm\(^{-2}\)), giving a current of 5.0 µA for 36 pads with a total area of 3.4 x 10\(^{-8}\) m\(^2\). This excluded 12 control pads that were not to be plated. The P-salt bath was used as before and at a five times greater concentration (80 mM (25.0 g·L\(^{-1}\))) in an attempt to reduce problems related to ion depletion at the pore bases. The gold chloride bath was 59 mM H.Au.Cl\(_4\).3H\(_2\)O operated at 21°C.

![Current Source](image.png)

**Figure 111.** Current source for IC electrodeposition. From the LM334 datasheet, a 5 µA current is set by adjusting the variable resistor to 13.4 kΩ. The input (V+) was connected to the Agilent 33220A waveform generator. The output of the generator was generally set to its maximum (10 V) to guarantee sufficient bias for the current source. The circuit was also designed to allow the option of −2 V (anodic) 2 ms pulses. The LM334 internal schematic [370] shows a reverse pulse can be driven through the device resulting in no damage and just a ~0.7 V drop across internal p-n junctions.

### 9.3.2 Results and Discussion

The current-controlled pulsed deposition scheme was observed to operate correctly. The P-salt bath then resulted in less gas evolution, but platinum deposition was poor, generally being limited to the periphery of the pads (Figure 112, Figure 113). Additionally, the problem of alumina dissolution at the pore bases had not been resolved (Figure 114).
Figure 112. IC array after deposition using current-controlled pulsed deposition. Deposits are not uniform and many pads appear to have deposits only at their peripheries. The marked pads are anodised controls which were unbiased during the plating of other pads. Pad diameters are 30 µm. IC19.

Figure 113. SEM top-view of IC pad (with FIB section) after current-controlled pulsed electrodeposition using P-salt. The bath has resulted in poor deposition, generally plating only at the pad periphery. Scale bar is 5 µm.
Gold deposition produced little visible gas, but deposits were also non-uniform across the array. The problem of sudden decreases in impedance was still observed, indicating that the pads were still being damaged.

9.4 Current Limitation Experiments

It was possible that the non-uniform deposition was caused by the impedance of the anodised pads varying across the array. As discussed above, it was plausible that the electrode with the lowest impedance at the start of deposition would have the greatest current density. Therefore most deposition would occur on this same pad, further lowering its impedance. This positive-feedback effect would therefore result in most of the current (5 µA) passing through a single pad instead of distributed across the 36 pads (139 nA per pad) and so result in damage. Further, this scenario would occur with both the voltage-controlled pulsed deposition scheme and the current-controlled scheme. It was therefore proposed to attempt plating individual pads whilst controlling current.
9.4.1 Methods

A Keithley 2400 SourceMeter was used to provide a constant current for electrodeposition of an individual anodised pad. To compensate for constant current (effectively 100% duty) instead of the 8–50% duty used previously, the current density was initially reduced, e.g. to 1 mA·cm$^{-2}$ (7 nA).

To extend the above constant current scheme to all pins, resistors were used in series with each pin (Figure 115). The SourceMeter recorded current and voltage versus time which enabled an appropriate value for the series resistor to be determined. From the 2400 SourceMeter tests, the potential across the electrode was measured to be small (typically 1.0 to 2.35 V for suitable pad plating currents of up to ~150 nA). A suitable resistor was therefore chosen from standard values using $R = \frac{(V_{33220A} - V_{pad})}{I}$. With a 68 M$\Omega$ resistor, the maximum current was estimated to be $I = (10 - 1.0) / 68 \times 10^6 = 132$ nA and the minimum current estimated to be $I = (10 - 2.35) / 68 \times 10^6 = 112$ nA.

Pulsed electrodeposition was re-introduced by using the Agilent 33220A waveform generator and the series resistor. Real-time EIS using the 4294A could then again be used to measure pin impedance versus time. The duty cycle was increased steadily: 7 mins at 10% duty, followed by 11 mins at 20% duty, 4 mins at 30% duty and 8 mins at 50% duty (a total of 30 mins).

P-salt and gold chloride baths were used as above. Additional baths tested were chloroplatinic acid (24 mM (1%) CPA H$_2$PtC$_1$_6.6H$_2$O with 264 µM (0.01%) Lead(II) acetate trihydrate) and a commercial gold cyanide bath (KAu(CN)$_2$ (pH ~5, Spa Plating, UK)). The P-salt bath was operated at 35$^\circ$C and the others at 21$^\circ$C.

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70 The oscilloscope could no longer be used to probe the circuit since the 'scope input impedance of 1 M$\Omega$ would have adversely affected the circuit operation.
Figure 115. Method for plating the IC electrode array by using current limiting. The circuit is an approximation to a pulsed current source. The Agilent 33220A output generates 10 V pulses at 8–50% duty. The 68 MΩ resistor is a pseudo constant current source since the potential dropped across the electrode is small compared to the 10 V source.

For simultaneous pulsed current of whole arrays, the above resistor circuit was duplicated for each pin. A printed circuit board was designed with ZIF socket and 68 MΩ surface mount device (SMD) series resistors, with DIL switches to enable switching of individual or groups of pins (e.g. to enable a control quadrant to be isolated).

Figure 116. The printed circuit board used for pulsed current plating of the whole electrode array. Each pin is connected via a DIL switch and series 68 MΩ SMD resistor (on the backside of the board). All pins are connected to the pcb header pins in the top left corner. It was necessary to keep the board clean due to contamination (e.g. flux and finger grease) affecting the value of the resistors.
9.4.2 Results and Discussion

Plating a single anodised pin using gold chloride with a constant current of 1 nA (1 mA·cm$^{-2}$) and 21 nA resulted in little visible change to the pad over 30 minutes. Plating at 45 nA (6.4 mA·cm$^{-2}$) resulted in the pad changing to a uniform orange colour, indicating successful deposition. Similar conditions were replicated using the 33220A waveform generator and 68 MΩ resistor circuit of Figure 115. Plating proceeded without gas evolution and the deposit appeared to be uniform (Figure 117). Real-time impedance measurements were performed, but results were affected by ambient electrical noise due to the high impedance of the single electrode (Figure 118).

Figure 117. Current controlled pulsed electrodeposition of a single electrode using gold chloride. The left pad is a control. The right pad (IC25, pin 11) has been plated for 30 mins with a pulsed current scheme (8% duty increasing to 50% duty at 22 mins). Pad diameter is 30 µm.
The conditions used for the successful single pin deposition (pulsed current, gold chloride, 68 MΩ series resistor) were duplicated for all pins using the printed circuit board. All pins were found to plate uniformly, with each electrode changing to an orange colour. The results are shown in Figure 119.

SEM analysis confirmed that plating was uniform across the area of each electrode, with gold correctly filling the pores (Figure 120). Most importantly, the porous alumina was intact, therefore maintaining the required corrosion resistance and allowing for future developments of other electrode design variations.
Figure 119. Deposition of gold using current pulse and individual series resistors. Images a–d were taken through the gold chloride solution and show the appearance after 6, 17, 22 and 29 mins of plating, respectively; e. After 30 mins. The image is slightly clearer than (d) as the plating solution has been removed and the array rinsed and dried. All pads appear to have plated uniformly. Pad diameter is 30 μm.
Figure 120. FIB sections showing successful deposition of gold into porous alumina: a. all high contrast material is gold which is filling the pores and creating a solid film above the porous layer. This was expected to be ideal for culturing neuronal cells as the surface of the electrode is almost flush with the passivation. The gold is somewhat dendritic which could be a result of its emergence from the pores, or the formulation of the basic gold chloride bath, or could be related to the pulsed current scheme. Branching at the pore bases is visible. This is expected with voltage ramp barrier oxide thinning. A small area of porous alumina under the surface of passivation is devoid of gold. This was not present on all pins and the reason for this is not understood. It is possibly related to lack of bath agitation or the blocking of pore tops by gold in adjacent pores. The empty porous alumina appears to be intact and therefore should continue to prevent corrosion in this region; b. a higher magnification image showing the filled pores with inter-linking. Branching at pore bases is again evident, as marked by the arrows. (Scale bars are 200 nm.)
A further IC was plated using the same conditions as above, except the plating sequence was interrupted for groups of pins: pins 1–5 were not plated (porous alumina controls), pins 6–8 were turned off using the DIL switches after 7 mins (at the end of a 10% duty) and pins 14–18 were turned off after 19 mins (during the 30% duty). The results are shown in Figure 121 and show that nucleation is initially patchy. However, by 19 mins nearly all pores have been filled and a gold layer is forming across the surface. If a variant of electrode design were to require a more uniform deposition, then this could form part of future work. Factors affecting uniformity may include plating bath formulation and bias scheme.

Figure 121. Sections showing electrodes with interrupted plating: a. and b. show deposition after ceasing deposition at 7 mins. Nucleation has not yet occurred at the base of all pores, even though some areas already have pores completely filled; c. and d. show an electrode with deposition ceased at 19 mins. Nearly all pores are completely filled with an additional solid gold film forming over the surface. (IC47, chloride bath. Scale bars for top views are 2 µm. FIB section scale bars are 200 nm.)

The commercial gold cyanide bath was evaluated as a comparison to gold chloride. Deposition using the gold cyanide was non-uniform (Figure 122) with an appearance similar
to ICs processed using the P-salt. The P-salt was again tested using the above conditions but the problems of poor and non-uniform deposition and gas evolution remained. The cause of these differences is not yet understood, but it was noted that the cyanide (pH ~5) and P-salt (pH 7) baths were less acidic than the gold chloride (pH 0.5). This warrants further investigation.

Figure 122. Gold cyanide plating: The electrode on the right side has been plated using a gold cyanide bath with all other conditions the same as for the electrodes plated with gold chloride. The gold has not covered the entire pad and has started to deposit beyond the pad periphery at its top-left corner. The pad to the left is a control. (IC25, pin 10. Pad diameter is 30 µm.)

The impedance of the electrode arrays successfully plated with the gold chloride bath was tested using the Solartron 1260A/1296. Figure 123 shows impedance magnitude, |Z|, per unit area and phase, \( \phi \), for IC arrays. Unprocessed CMOS pads showed a predominantly capacitive characteristic (i.e. \( \phi < -70^\circ \) except at low frequencies) which is due to the double layer capacitance. Anodising and electrochemically thinning can be seen from the figure to reduce impedance magnitude, with a further reduction in impedance resulting from the gold deposition. The phase indicates the electrode characteristics become less dominated by the double layer capacitance after gold deposition. Therefore, both the porous alumina and gold IC electrodes provide electrical performance better than the aluminium metal surface but with the benefits of being resistant to corrosion and biocompatible.
Figure 123. Impedance magnitude, $|Z|$, per unit area and phase, $\phi$, for an IC array at various stages of processing: unprocessed CMOS pads (‘Aluminium’); after anodising and thinning (‘Porous alumina’); and after gold deposition using the chloride bath (‘Gold’). Data are for a 48 electrode array, each electrode being 30 µm diameter. The individual electrode impedance, $|Z|_{pad}$, is shown on the second y-axis for reference.

9.5 Platinum Black

The impedance of the gold electrode is expected to be suitable for most applications. However, as discussed in Section 2.9.3, neuronal recording applications require a minimised impedance to reduce thermal noise and maximise the signal-to-noise ratio. Commercial MEAs used for mammalian neuronal recordings include the Multi Channel Systems and
MED64 (Alpha Med Scientific, Inc.) products. Quality assurance data for MEAs purchased from Multi Channel Systems showed all electrodes had individual impedances of 39 k – 41 kΩ at 1 kHz (the characteristic frequency of an action potential) which is in agreement with their documentation claiming 20 k – 400 kΩ for their range of titanium nitride electrodes [255]. The MED64 electrodes are claimed to have impedances of 7 k – 10 kΩ at 1 kHz [198]. To prepare CMOS IC electrode arrays for neuronal recordings it was decided that the 1 MΩ impedance at 1 kHz achieved with the gold deposition (Figure 123 above) was inadequate. The standard technique of lowering the impedance by coating with platinum black was to be used, with a target impedance of 40 kΩ (at 1 kHz) for the 30 µm diameter electrodes.

Operating a chloroplatinic acid bath at high current density (e.g. 100 mA·cm⁻²) achieves the desired dendritic and porous growth of platinum black, as opposed to the compact platinum deposition achieved at low current densities (5–10 mA·cm⁻²). A summary of methods is given in Table 14. These are intended for various applications, including platinum tip electrodes and electrochemistry. Therefore not all conditions listed are expected to be suitable for planar CMOS electrodes since it is desirable to maintain the planar surface for optimum plating of cells. This was discussed in Section 2.9.5 and illustrated by contrasting Figure 27 with Figure 28: it was questioned whether the MED64 electrodes with tall (7.39 µm) dendritic growth of platinum black above the substrate would cause problems with cell plating.
Table 14. Conditions used to deposit platinum black.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Plating solution</th>
<th>Operating conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schuettler et al. (2005) [249]</td>
<td>1.4% CPA, 0.02% Lead(II) nitrate Pb(NO₃)₂</td>
<td>250 mV, 10 s, ultrasound agitation</td>
</tr>
<tr>
<td>Mills (2006) [247]</td>
<td>3% CPA, 0.025% lead acetate, 1% conc. hydrochloric acid</td>
<td>≈5 mA cm⁻²</td>
</tr>
<tr>
<td>Werdich et al. (2004) [252]</td>
<td>2.5% CPA, 0.05% lead acetate</td>
<td>400 mA cm⁻², 10–20 s max.</td>
</tr>
<tr>
<td>Mathieson et al. (2004) [371]</td>
<td>1% CPA, 0.08% lead acetate</td>
<td>400 mA cm⁻², 20 s</td>
</tr>
<tr>
<td>Morrissey (2000) [372]</td>
<td>2% CPA, 30% HCl,</td>
<td>65°C, 10–200 mA cm⁻²</td>
</tr>
<tr>
<td>Borkholder (1998) [373]</td>
<td>1% CPA, 0.01% lead acetate</td>
<td>500 mA cm⁻², ultrasonic agitation</td>
</tr>
<tr>
<td>Rao and Trivedi (2005) [374]</td>
<td>0.5–2.5% CPA, 18–30% v/v HCl</td>
<td>45–90°C.</td>
</tr>
<tr>
<td>Ilic et al. (2000) [375]</td>
<td>3.5% CPA, 0.005% lead acetate</td>
<td>1.4 V for 190 s or 30 mA cm⁻² for 140 s.</td>
</tr>
<tr>
<td>Johnson et al. (2005) [376]</td>
<td>“Kohlrausch’s Solution” (3% CPA, 0.025 N HCl)</td>
<td>20 mA cm⁻² 600 s</td>
</tr>
<tr>
<td>Sawyer (1974) [377]</td>
<td>3.5% CPA, 0.005% lead acetate</td>
<td>30 mA cm⁻², 5 mins, stirring</td>
</tr>
<tr>
<td>James et al. (2004) [378]</td>
<td>Solution No. LC18680-7, LabChem Inc., U.S.</td>
<td>400–500 mA cm⁻², 30–90 s</td>
</tr>
<tr>
<td>Fischer and Weimer (1964) [379]</td>
<td>CPA as 1.0–2.5% Pt metal, 1.0–39% HCl, pH &lt; 2.2</td>
<td>45–75°C, 38 mA cm⁻²</td>
</tr>
</tbody>
</table>

9.5.1 Materials and Methods

CMOS ICs with porous alumina and gold electrodes were prepared as discussed in Section 9.4 above. Platinum black electrodeposition was performed using the standard chloroplatinic acid bath (24 mM (1%) CPA H₂PtCl₆·6H₂O with 264 μM (0.01%) Lead(II) acetate trihydrate) operated at 21°C. Using Table 14 as a guide, 100 mA cm⁻² was initially chosen to encourage deposition as platinum black but avoid large out-growth from the defined pad area.

Preliminary experiments were performed using single IC electrodes. A current density of 100 mA cm⁻² was produced by 50 V d.c. from the 2400 SourceMeter through a 70 MΩ resistor to give 714 nA across the 30 μm diameter electrode. An iBasic program for the 4294A analyser was used to control the duration of the plating (Appendix E), allowing consecutive plating pulses, between which EIS readouts were taken. Suitable plating
conditions were then transferred to the pcb with 68 MΩ series resistors for simultaneous deposition onto all 48 electrodes.

9.5.2 Results and Discussion

Impedance versus time for deposition of individual pins at 100 mA·cm⁻² is shown in Figure 124. The default plating pulse width used in the iBasic program was 5 s. This resulted in increasing impedance with no visual indication of plating. The reason for this was not understood, but it was speculated that the electrochemical dynamic equilibrium necessary for deposition cannot be achieved within 5 s and that other chemical processes may have dominated during each 5 s plating pulse. After 30–40 s, the pulse width was increased by 10 s which resulted in a rapid decrease of impedance and a blackening of the electrode (Figure 125). This was accompanied by profuse evolution of gas.

![Figure 124. Impedance, |Z|, versus time, t, for Pt-Black deposition of individual pins (EIS data for f = 2.3 kHz, deposition current density = 100 mA·cm⁻²). EIS readouts were performed using the 4294A. It can be seen that pulses of 5 s (before t = 40 s) increased rather than decreased the impedance. Setting the 4294A program to generate 10 s pulses rapidly decreased the impedance: pin 10 impedance decreased by a factor of 32 (to 6.35 kΩ at t = 147 s) and pin 11 by a factor of 25 (to 6.40 kΩ at t = 112 s). Unlike other |Z|(t) graphs shown for 110 Hz, data are shown here for 2.3 kHz as this is closer to the 1 kHz characteristic frequency of action potentials. It should be noted that these values do not include the bath solution resistance due to the system compensation feature of the 4294A (which is not available on the Solartron 1260A/1296).

71 A facility to manually select 5 s (default), 10 s or 15 s pulses was made available.
Impedance spectroscopy using the Solartron 1260A /1296 confirmed the large reduction in impedance to 60 kΩ at 1 kHz observed using the 4294A (Figure 126). This is comparable to the 40 kΩ of the Multi Channel Systems TiN electrodes of the same dimension [196]. SEM analysis of the platinum black deposition is shown in Figure 127. This shows the platinum black had grown to a height of approximately 2–3 µm above the level of the passivation surface. For reasons explained above, it was considered that this could be detrimental to the plating of neuronal cells onto electrodes.
Figure 126. Impedance spectroscopy for platinum black deposition (IC25, pin 11). Gold and porous alumina (AAO) pins are shown for reference. At $10^{-2}$ Hz the platinum black has reduced the impedance by a factor of 319 versus the gold electrode. At 1 kHz the platinum black has reduced the impedance by a factor of 91 to 60 kΩ.
Due to the above limitations, an attempt was made to produce a more controlled rate of deposition and more compact platinum black layer by reducing the current density. The 48 electrodes of CMOS arrays were plated simultaneously through 68 MΩ resistors for 20 s at 50 mA·cm$^{-2}$ (25 V from the 2400 SourceMeter), after which the impedance at 1 kHz was noted using the 4294A. ICs with average electrode impedance greater than 40 kΩ were plated for further 20 s periods. All ICs had average electrode impedances of ≤40 kΩ within 60 s. The results for a selection of ICs are given in Table 15. This illustrates how the impedance falls rapidly, with a few ICs having very low impedances of only 2–3 kΩ per electrode. This reflects the difficulty in controlling the precise duration required for rapid deposition of platinum black: the experiments showed how a succession of 5 s pulses did not produce any deposition whereas periods of 20 s at 50 mA·cm$^{-2}$ provided poor control for adjusting the impedance. However, visual analyses of each IC and SEM analysis (Figure 128) showed the process control was adequate to prevent the large out-growth of platinum black.

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72 These measurements required removing the IC from the pcb since impedance could not be measured through the 68 MΩ resistors.
seen in the initial trials and the target of \(<40\,k\Omega\) per electrode was readily achievable and reproducible for all ICs.

<table>
<thead>
<tr>
<th>IC reference number</th>
<th>Number, (n), of 20 s plating periods required to reduce the average electrode impedance to (&lt;40,k\Omega).</th>
<th>Average electrode impedance (k(\Omega)) after 20(n) seconds of plating.</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>1</td>
<td>42.0</td>
</tr>
<tr>
<td>38</td>
<td>3</td>
<td>2.55</td>
</tr>
<tr>
<td>39</td>
<td>3</td>
<td>3.39</td>
</tr>
<tr>
<td>40</td>
<td>1</td>
<td>37.8</td>
</tr>
<tr>
<td>41</td>
<td>2</td>
<td>30.0</td>
</tr>
<tr>
<td>42</td>
<td>2</td>
<td>23.2</td>
</tr>
</tbody>
</table>

Table 15. Impedances for electrodes of six ICs after deposition of platinum black using a series of 20 s plating periods.

The final electrode EIS results are shown in Figure 129. In addition to the lowering of impedance magnitude, the phase indicates the electrode characteristics become less dominated by the double layer capacitance during processing, with the platinum-black showing more
significant resistive elements: at high frequencies the impedance magnitude of the double layer becomes sufficiently low to allow the solution resistance to start to dominate the system, as illustrated by the reduced magnitude of $d|Z| / df$ and the increased phase angle.

![Graph showing impedance magnitude and phase for different electrode types](image)

Figure 129. Impedance magnitude, $|Z|$, per unit area and phase, $\phi$, for an IC array with platinum black electrodes (c.f. Figure 123): unprocessed CMOS pads (‘Aluminium’); after anodising and thinning (‘Porous alumina’); after gold deposition (‘Gold’) and after platinum black deposition (‘Pt-Black’). Data are for a 48 electrode array, each electrode being 30 $\mu$m diameter. The individual electrode impedance, $Z_{pad}$ is shown on the second y-axis for reference.
9.6 Conclusions

Using the techniques developed in Chapters 7 and 8, it has been demonstrated that aluminium CMOS microelectrodes can be made biocompatible by converting them to porous alumina.

ICs were successfully anodised and their barrier oxides electrochemically thinned, resulting in impedance comparable to the unmodified aluminium and other planar electrodes. The alumina had inter-connected pores, as predicted by the earlier work using Al-Si-Cu coverslips.

It was shown that infiltrating the alumina pores with metal further reduced impedance. For applications requiring a planar electrode surface, gold was electrodeposited into the porous alumina to provide a bio-inert surface. This was achieved by scaling the voltages and currents of the coverslip pulsed electrodeposition scheme for the microelectrodes. It was discovered that a key requirement for uniform deposition across the array was to control current for each individual pin. It was shown that the plating bath formulation is critical to success: platinum P-salt and gold cyanide baths gave poor results, with uniform deposition achieved using a gold chloride bath. To understand these differences requires further research, but it was speculated that it may relate to pH and plating current efficiency.

For neuronal recording applications that often call for particularly low impedances, the planar gold was coated with platinum-black resulting in a further reduction in impedance to less than 40 kΩ (at 1 kHz) for each 30 µm diameter electrode.
Key Points

- For the first time, biocompatible CMOS electrode arrays were produced using low-cost post-processing.
- A pulsed current scheme was developed to enable electrodeposition of noble metal uniformly across the entire electrode array.
- Formulation of the plating bath is critical in avoiding damage and achieving a uniform deposition across the area of an electrode.
- Platinum black deposition has been controlled to simultaneously give a planar electrode surface and low impedance suitable for neuronal recordings.
- The manipulation of porosity and the electrodeposition of metal into pores demonstrate the flexibility of the process as a foundation for many variants of electrode design.
10 Neuronal Recording Application

10.1 Introduction

It is recalled that the original premise for this work was to develop electrodes for biological applications from CMOS technology, focussing on the most demanding application, i.e. neuronal recordings. In Chapter 3 a model of a plain porous alumina electrode was proposed for this application. The model assumed straight pores linking the underside of the cell and the titanium base of the electrode. However, for CMOS metallisation, it has been shown that the pores are not straight but interlinked, possibly resulting in loss of signal and, secondly, that the impedance of the plain porous alumina electrode – despite being slightly lower than the impedance of plain aluminium – might result in a poor signal-to-noise ratio. It was therefore proposed that lowering the impedance using platinum black (to ~40 kΩ at 1 kHz) might be beneficial. A low impedance porous alumina, gold and platinum black CMOS electrode has been developed for this purpose.

However, the concept of using a plain porous alumina electrode for neuronal recordings has not been neglected and it has been shown that a substrate with large alumina pores might present an advantage in terms of cell-substrate adhesion. Given that the principal element contributing to the electrode impedance is the double layer at the solid-solution interface and not the electrode materials (barrier oxide and titanium) it remains unclear what the total thermal noise generated by the electrode would be. A negative impact caused by interlinking pores has been speculated but this has not yet been investigated. As a comparison to the interlinked alumina pores, one may consider the platinum black interface: it seems unlikely that there can be a continuous tight cleft between the cell membrane and dendritic platinum black and so there will be conducting medium between the platinum black ‘branches’. Therefore platinum black electrodes also have lateral leakage paths that could cause signal degradation, yet they are used successfully for neuronal recordings [129],[193],[252],[371],[378],[380]. TEM imaging of the cell-substrate interface of a platinum black recording electrode would assist this work and could form part of future research. Additionally, it should be possible to deposit platinum black into the base of

73 See Section 2.9.3 for an overview of thermal noise.
alumina pores and so gain the benefits of enhanced cell-substrate adhesion but with optimal impedance. This also could be considered for future work. However, before evaluating these more advanced configurations, it was decided to first demonstrate the basic ability to record action potentials using CMOS by using the optimised gold and platinum black electrode array. This would be the first demonstration of neuronal recordings using a standard CMOS process.\textsuperscript{74}

\textbf{10.2 Materials and Methods}

Previous work at King’s College London had demonstrated neuronal recordings using NG108-15 mammalian neuronal cells and rat primary neurons (dorsal root ganglia) on MEAs from Multi Channel Systems. These devices had degraded with use and storage and so were no longer available as controls, but a similar experimental protocol was used here for testing the CMOS electrodes.

CMOS ICs were post-processed as described in Chapter 9. Thirteen ICs had been prepared with porous alumina and gold, coated with platinum black extending 2–3 \( \mu \)m above the passivation surface. Optimisation of the platinum deposition process yielded a further eleven ICs with the platinum black planar to the passivation surface. Prior to plating with cells, ICs were sterilised for 30 mins in ethanol, dried and coated with poly-l-lysine (poly-l-lysine (PLL) hydrobromide 15–30 kDa, 0.01\% w/v (P7890, Sigma-Aldrich, UK)), incubated for 1 hour at 37\(^\circ\)C, then rinsed with growth medium.

NG108-15 cells were cultured as described in 5.3.1, plating out onto ICs with a seeding density of 30,000–60,000 ml\(^{-1}\) (~500 \( \mu \)l per culture chamber) and incubated at 37\(^\circ\)C, 10\% CO\(_2\). After 24 hours, the additional step of differentiation was required to express the neuronal characteristics of NG108-15 cells. This was performed by adding isobutylmethylxanthine (IBMX) and prostaglandin E1 to the growth medium. Prior to recording, cells were incubated for a further period of either 6 or 13 days, refreshing the plating medium every 3–4 days.

\textsuperscript{74} i.e. a process not requiring additional semiconductor fabrication steps such as photolithography (c.f. Figure 25).
Dorsal root ganglion cells were dissected from adult rat\textsuperscript{75} and suspended in 500 µl of culture medium (DMEM with GlutaMax-II (Gibco), 10% FCS, 10 units.ml\textsuperscript{-1} Penicillin and 0.1 mg.ml\textsuperscript{-1} Streptomycin (Sigma-Aldrich), 50 ng.ml\textsuperscript{-1} nerve growth factor (NGF)). 15 µl was plated into the well of each IC (previously coated with PLL) and incubated for 30–60 mins (37ºC, 5% CO\textsubscript{2}) to allow the cells to start adhering to the electrode array. A further 350 µl of culture medium was then added to the culture chamber of each IC and incubated for a further 24 hours prior to recording.

Recordings were made using a programmable gain amplifier (PGA64, MEA1060, Rev C, Multi Channel Systems (MCS)) connected to a PC with data acquisition card (MCS) and ‘MC-Rack’ software (MCS) as shown in Figure 130. The data sampling rate was 25 kHz. The PGA was set to a gain of 500 and software filters used to reduce noise (low pass filter: 5 kHz cutoff for NG108-15s; 3 kHz cutoff for DRGs) and remove d.c. offsets and drift (2 Hz cutoff high pass filter).\textsuperscript{76} A platinum wire electrode was used in the culture chamber to ground the recording buffer. Recordings were performed at room temperature, initially using the culture medium which was then replaced using a superfusion of recording buffer. The recording buffer for NG108-15s was as stated in Section 5.3.1.2. The DRG recording buffer was distilled water with 130 mM NaCl, 3 mM KCl, 1.0 mM MgCl\textsubscript{2}, 11 mM glucose, 5 mM HEPES, 1.0 mM CaCl\textsubscript{2}, with pH adjusted to 7.4 using HCl or NaOH. To depolarise the cells, arrays not showing spontaneous action potentials were perfused with buffer containing 15 mM potassium chloride instead of 3 mM.\textsuperscript{77}

To verify spontaneous action potentials, spikes were blocked using 100 nM TTX (tetrodotoxin citrate, Tocris Bioscience, UK, P/N 1069) in buffer. TTX selectively blocks sodium ion channels and the process is reversible by thorough washing. To increase the rate of spikes, 100 µM ATP (Sigma-Aldrich, P/N A2383; c.f. Section 2.1.3) was perfused.

\textsuperscript{75} Dissection was performed by Dr Reg Docherty, King’s College London.

\textsuperscript{76} The PGA hardware has a bandwidth of 5 kHz (1 to 5000 Hz).

\textsuperscript{77} From the Nernst Equation, $E_K=58 \log_{10}[K_0]/[K_{in}]$. Therefore, to depolarise from −60 mV at 3 mM KCl to 0 mV would require ~30 mM KCl (at 25ºC). Similarly, to depolarise to the action potential threshold of approximately −40 mV requires ~15 mM KCl.
Figure 130. Neuronal recording apparatus: a. programmable pre-amplifier, PGA (i) connected to power supply (ii) and PC (iii). The PGA is housed in a Faraday cage (iv) to shield the small signals from ambient noise. Gravity fed media were perfused across the electrode arrays using up to four media held in syringes (v), selectable using a rotary switch (vi). Used media were removed from the IC culture chamber using a pipe and tubing connected to a syringe pump (vii). Cage, pump, superfusion tubes and PGA were connected to a bus bar to form a common ground (viii). b. An enlarged view showing PGA (i) and ZIF socket with IC (ix). The IC is in the process of being set up, with only the perfusion removal pipe and electrode in place. The micromanipulator (x) is used to position the superfusion feed tube (not shown).

10.3 Results

No spontaneous action potentials were recorded for ICs plated with NG108-15 cells (n = 23 over 3 runs for ICs tested after 6 days in-vitro (DIV); n = 49 over 4 runs for ICs tested after 13 DIV). Depolarising the cells with KCl did not induce activity.

From two runs of ICs plated with rat DRGs (n = 42) a single electrode of one IC displayed a spontaneous action potential in culture medium. The raw voltage versus time data of the recording is shown in Figure 131. This example shows 6 action potential ‘spikes’ which are superimposed on the noise. The inset has an expanded time axis showing the shape of the action potential. Microscope images of the pin 35 recording site (Figure 132) show the cell partially covering the electrode.

The effects of superfusion with TTX are illustrated by Figure 133. This shows an initial period of spontaneous firing of the neuron up to 43 s, with a spiking frequency of 0.5–3 Hz. TTX superfusion was started at 43 s and, after a short delay for the TTX to travel along the tubing and into the culture chamber, it caused spiking to cease. TTX was washed at ~110 s and, after a delay of ~913s, the spontaneous firing recommenced. The effects of ATP and KCl on spontaneous firing are illustrated by Figure 134. These graphs show the spike frequency...
before (control), during and after (wash) superfusion with KCl and ATP. Statistical analysis (ANOVA) of these data confirmed a significant (p<0.001) increase in spike frequency during superfusion. These superfusions of TTX, ATP and KCl had the effects attributable only to neuron activity, therefore validating the observed spikes as action potentials.
Figure 131. Raw data of the recorded action potential (pin 35, IC39). The main image shows 6 action potentials with voltage (µV) versus time (ms). The expanded view shows details of a single action potential with voltage (µV) versus time (s).

Figure 132. a. Microscope image showing the rat DRG on the electrode connected to pin 35 (marked) of IC39 which resulted in a recording of an action potential. The cell appears larger than the electrode diameter (30 µm). Only a few cells with similar appearance are present on the array (visible mainly to the right side of the array). High contrast areas are clumped cells above the IC surface; b. An enlarged image of pin 35 and DRG. The cell appears to be only partly covering the electrode. The poor image resolution is caused by imaging through the recording buffer.
Figure 133. Spike frequency versus time, \( t \). The sample period (epoch) is 1 s. The neuron fires with a frequency of 13-16 Hz in recording buffer at \( t < 44 \) s. Superfusion with 100 nM TTX starts at 44 s and, after a short delay, the spiking stops by \( \sim 73 \) s. TTX washing begins at \( \sim 136 \) s and, after a long delay, spontaneous firing recommences at \( \sim 1022 \) s.
Analysis of the voltage versus time data showed the characteristic frequency of the single recorded neuron was 1.1 kHz (Figure 135a). This confirmed that the 1 kHz used for characterisation of electrode impedance in previous chapters was appropriate. The validity of the FFT was confirmed by analysing the same data file during a period where TTX had been used to suppress the action potentials (Figure 135b, c). Further, it was shown that the noise was 9.3 $\mu$V$_{\text{rms}}$ and the mean peak action potential amplitude was 76.1 $\mu$V, giving a signal-to-noise ratio of 8.2. In [129] it is stated that typical noise for similar metal recording electrodes is 5–10 $\mu$V and that typical spike amplitude is 500 $\mu$V for mammalian neurons (giving a signal-to-noise ratio of 50–100) and 2 mV for cardiomyocytes. The lower than typical spike amplitude achieved with the CMOS MEA may be partly attributable to the neuron only partially covering the electrode. This was discussed in Section 2.5.2 and is addressed in the following section.
Figure 135. Fast Fourier transforms (FFTs) of recordings, shown as r.m.s. of the amplitude versus frequency: a. the FFT of a single action potential. The spectrum shows the spike to have a characteristic frequency of ~1.1 kHz (marked). The resolution of the FFT is limited by the number of data points in the single spike which is determined by the 25 kHz sampling frequency of the MCS data acquisition card; b. in the absence of action potentials (suppressed with TTX) the spectrum shows only the noise, shown here on the same scale as (a); c. the same FFT spectrum as (b) except shown on an expanded y-axis.

10.4 Discussion

The recorded action potential validates the suitability of the electrode design for neuronal recordings. With reference to Section 2.9.1 and Table 3, the recording demonstrates an improvement over the 0% success rate for previous work using unmodified CMOS. Additionally, the ability to record after a cumulative 56 days in vitro (4 runs) demonstrates longevity of the electrode. It is impractical to define a success rate based on a single recording, but it is unlikely the single CMOS recording represents a better success rate than...
the 18.5% achieved using the MCS MEAs. A larger sample size is therefore required and is already being planned as future research. In preparation for this work, other factors for consideration include:

i. Electrode area, pitch and number of electrodes: The present ‘WET4’ design of the CMOS MEA has only 48 electrodes compared to the 64 electrodes of the MCS MEA. This would account for a 25% reduction in successful recordings using the CMOS MEAs. Decreasing the electrode area may increase the signal amplitude when fully covered by a cell (Section 2.5.2). However, this must be offset by the reduced probability of cells covering smaller electrodes. Increasing the number of electrodes per array would require a different package or require on-chip multiplexing circuits.

ii. Protein coatings: Problems with the PLL coating may lead to poor adhesion and lack of cell spreading on the passivation and electrode surfaces. A review is provided in [62] which may be useful for improving the protein coating protocol.

iii. A problem with the NG108-15 cell culture might have resulted in poor expression of ion channels (e.g. a problem with cell differentiation).

iv. Cells might be reluctant to settle on an electrode surface raised above the passivation surface: in Section 9.5.2 it was shown how the platinum black may form a ‘mound’ above the IC surface and it is therefore conceivable that cells settling under gravity may move off these raised electrode areas onto the surrounding IC passivation. It might therefore be necessary to increase the number of CMOS ICs which are processed to have platinum black planar to the passivation surface. It is noted however that the MED64 platinum black electrodes are far from planar (Figure 28) but claim to produce successful recordings.

v. Cell adhesion to silicon nitride IC passivation has been proven and is accepted as adequate (See Section 2.9.2 and references therein). However, further research is required to understand if the chemistry of silicon nitride might under any circumstances (e.g. with patchy protein coating) be detrimental to cell adhesion or neuronal cell activity.

78 Using Student’s t-test to compare CMOS (1/42=2.4%) and MCS MEA (18.5%) success rates, with n = 42, means of 0.024 and 0.185 respectively, $\sigma^2 = 0.023$ and $\alpha = 0.05$, it is unlikely ($p>0.999$) that the 1/42 recording represents a better success rate than the MCS electrodes.

79 The MCS amplifier has only 64 channels. An array with more than 64 electrodes would require multiplexing onto these 64 channels using a switching circuit.

80 As for many clonal cell lines, NG108-15 cells do not properly express their neuronal characteristics during growth. This is similar to stem cells. The process of differentiation causes a change in gene expression and for the NG108-15 this results in the cell becoming more neuron-like, with neurite extensions and an increase in ion channel density.
vi. It is possible that spikes were missed due to the poor signal-to-noise ratio. Redesigning the hand-wired ZIF socket pre-amplifier interface board might reduce noise by a factor of 2 (e.g. by designing a pcb with ground plane and improved screening).

vii. Platinum black coatings may degrade with use [182]. Further EIS experiments would determine if the electrode impedance degrades over time, in which case the platinum black may require ‘refreshing’ between uses.

viii. Positive controls: benchmarking against MCS MEAs would assist the diagnosis of the poor recording success rate.

10.5 Conclusions

The CMOS electrode array has been proven to be non-invasive and to record spontaneous and evoked action potentials over several hours. The ability to modulate cell activity in a pharmacological manner was demonstrated using TTX, KCl and ATP.

The recording success rate was lower than achieved in previous work using Multi Channel Systems MEAs and this needs to be understood through further work. A range of possible factors have been identified and are mostly related to the quality of cell plating, e.g. differentiation of NG108-15s or cell adhesion and spreading.

Key Points

- Neuronal recordings using a low-cost post-processed CMOS electrode have been demonstrated for the first time.
- The longevity of the CMOS post-processing design was demonstrated by repeated use of the electrode array to 56 days.
- The neuronal recording rate was poor and requires further work to understand the causes.
11 ECIS Application

11.1 Introduction
The post-processing CMOS electrode technology can be employed for many applications. Its principle function to record neuronal action potentials has already been demonstrated. The same electrode array design can also be used as an impedance sensor (ECIS, Section 2.6) even though the ‘WET4’ design was not optimised for this purpose: the combined area of 48 electrodes of 30 µm diameter electrode (0.03 mm²) senses only a small proportion of the exposed IC substrate area (~1 mm²), whereas larger electrodes may give improved performance. ECIS is most suited to cells that form confluent films with tight cell-cell junctions as these produce an unbroken high impedance film that has the greatest effect on the measured impedance [131]. A suitable cell line, Caco2 (human colon epithelial adenocarcinoma) [381], was available at King’s College London, cells donated courtesy of Prof. Helen Cox.

11.2 Methods and Materials
The 48 electrodes of ‘WET4’ ICs were used as a single impedance sensor by connecting together all pins of the package. A silver chloride (Ag/AgCl) wire electrode was suspended in the culture medium of the IC culture chamber to provide an electrical ground. Pins of the IC and wire electrode were connected to the two terminals of an Agilent 4294A Precision Impedance Analyzer (Figure 136). As experiments were to be conducted over 4 days, the rate of EIS readouts was reduced after 15 mins and again after 1 hour in order to reduce stored data file size and to capture any short-term processes immediately after cell plating. This was controlled using an iBasic program (Appendix F). For each readout, EIS data were stored for 40 Hz to 1 x 10⁶ Hz, although it has already been established that an optimum frequency for detecting cell-substrate impedances changes is 40 kHz [131].
Caco2 cells were cultured in 50 ml flasks, each containing 5 ml of culture medium (Dulbecco’s Modified Eagle’s Medium/Nutrient Mixture F-12 Ham (Sigma-Aldrich, D6421) and supplemented with 1% w/v L-glutamine, 10% v/v FCS and 1% w/v nonessential amino acids) and incubated at 37°C, 5% CO2 [382]. Passaging was performed every 3–4 days at 90–100% confluency. To dissociate cells from flasks and each other, the culture medium was removed from the flasks, the cells rinsed in ~3 ml of versene buffer (10 mM EDTA in calcium-free and magnesium-free phosphate-buffered saline) and then trypsinized by adding 1.5 ml of trypsin (0.5% w/v, Worthington, TRL3, filter sterilised) and incubated for 3–6 mins. The trypsin was neutralised by adding ~5 ml of culture medium. Cells were detached by tapping the flasks, centrifuged for 5 mins at 700 rpm and split 4:1 or 5:1 into 5 ml of fresh culture medium. Cells were similarly trypsinized to detach them from ICs after each run of the ECIS experiment.

ICs fabricated with porous alumina, gold and platinum black electrodes (Chapter 9) were prepared by sterilising in ethanol for 30 mins. Approximately 500 µl of cells were plated into each IC culture chamber after passaging but without splitting. Cells were incubated for 3-5 DIV at 37°C, 5% CO2 whilst recording impedance.
11.3 Results and Discussion

The results are shown in Figure 137, illustrating the impedance of the combined 48 electrodes versus time for ICs with and without cells. It shows that the Caco2 cells have little effect on impedance until \( \sim 10^5 \) s (\( \sim 28 \) hours). It is assumed that only after this time have cells adhered, divided and formed tight cell-cell junctions. The data were fitted to a sigmoid as the impedance is expected reach a plateau once the electrodes are covered by a confluent layer of cells. ICs with only culture medium (without cells) show little change (<200 \( \Omega \)) in impedance during the experiment.

Figure 137. ECIS results showing magnitude of imaginary impedance, \( |Z|_{\text{imag}} \), versus time, \( t \). The main graph shows individual data points for controls (growth medium without cells, \( n = 3 \)) and Caco2 cells (\( n = 4 \)). Fitted curves are shown for both sets of data (sigmoid fit with 95% confidence intervals (C.I.). The fitted curve for the controls overlaps the C.I. lines which therefore are not visible). For clarity, the inset shows only the fitted sigmoid curves. All impedance data is for 47.9 kHz at 37°C.
The capacitance, $C$, of the electrodes is derived simply from EIS data using $C = 1 / (2\pi f |Z_{\text{imag}}|)$, with frequency, $f$, and where $Z_{\text{imag}}$ is the imaginary component$^{81}$ of impedance, $Z$. A model is presented in [131] showing an almost linear relationship between capacitance and fraction of electrode surface covered by cells: with 1.0 being the normalised capacitance of an uncovered electrode, completely covering an electrode with cells results in a normalised capacitance of 0.3. Figure 138 shows the capacitance for a single IC with Caco2 cells, exhibiting a reduction in normalised capacitance from 1.0 to 0.4. Using the linear relationship, this represents electrode coverage of 86%. It is also noted that the capacitance stabilised at 3 days which is probably indicative of the cells becoming confluent.$^{82}$ These results are therefore in broad agreement with the model. Unfortunately, it was not possible to correlate electrical results with images of the electrode array since the epi-illuminated microscope was incapable of imaging the flattened monolayer (the cells appearing transparent).

$^{81}$ The imaginary component of the complex impedance represents the capacitive (and inductive, if present) elements of a system. See Section 2.6. The 4294A automatically calculates $|Z|_{\text{imag}}$.

$^{82}$ It is also for this reason that a sigmoid was used for curve fitting.
Figure 138. Electrode capacitance, $C$, versus time for a single IC with Caco2 cells ($f = 47.9$ kHz). The change in capacitance from 2.5 nF to $\sim$1.0 nF represents a change in normalised impedance from 1.0 to 0.4, respectively. The initial increase in capacitance during the first hour was observed for ICs with and without cells, suggesting the effect may be caused by a change at the solid-solution interface, e.g. wetting of the platinum black.

11.4 Conclusions

The experiment demonstrates the ability of the low-cost post-processed CMOS IC technology to function as an impedance sensor which can be used to study tumour growth, wound healing, cell migration and to understand how cells interact with a substrate. Furthermore, re-use of the ICs demonstrates the corrosion resistance of the modified electrodes and the stability of the IC packaging to at least 40 days.

Further work could develop packaging and on-chip IC circuits for commercial ECIS applications. For example, ICs placed in wells of microtitre plates would dispense with the large number of electrical connections currently required to connect passive electrodes. This
would enable measurements to be made in real-time and would avoid removing the cells from the incubator where sterility, temperature and humidity conditions are ideal.

**Key Points**

- Cell-substrate impedance sensing using low-cost post-processed CMOS electrodes has been demonstrated for the first time.
- The durability of the CMOS post-processing design was again demonstrated, with use extending to 40 days.
- Inclusion of on-chip CMOS circuits would enable commercial applications in drug discovery and for research.
12 Conclusions and Outlook

The motivation for this work was to contribute to advancements in neuroprosthetics, drug discovery and cell-based biosensors. Central to these applications is the need for bi-directional transfer of information between biological and electronic parts of a system. A review of these fields showed that research into biosensors commenced with vigour only in the 1980’s. Commercial neuroprosthetics have appeared more recently but are generally limited to stimulation of nerves. There is a notable absence of applications that transfer information out of the nervous system at the scale of the individual neuron, where electronics must detect and record the small ‘action potential’ signals. The exploitation of neuronal cell excitability is also absent in the field of drug development where methods still predominantly rely on optical detection using cells altered to include labels that fluoresce. Patch clamp is the established method in the research lab but it is difficult to scale up from single neuron recordings and is invasive. Some progress has been made in drug discovery by developing ‘label-free’ systems that can, for example, measure cell growth using electric cell-substrate impedance sensors (ECIS) or automate patch-clamping using specialised micro-fluidic substrates, but such systems are not conducive to the high-throughput screening methods which have become standard in the industry. Environmental and defence applications such as detection of pollutants, bacteria and other toxins may also benefit from cell-based biosensors as these promise much faster measurement than the present laboratory culture and analysis methods.

IC technology is readily available for biosensors in its ubiquitous form as complementary metal oxide semiconductor (CMOS). However, to create a suitable interface to biological cells the biosensor must include electrodes that are non-invasive and biocompatible – requirements that CMOS does not meet. Researchers have therefore modified CMOS ICs by applying additional layers, but the only method proven to establish biocompatibility requires the use of microfabrication equipment in a semiconductor cleanroom. This is suitable for ICs manufactured for research purposes but, due to the high cost of this approach, the economies of scale provided by CMOS are lost. If CMOS biosensors are to be commercialised, then a low-cost method to modify the technology is required. Development of such a process was the overall objective of this work.
In considering CMOS post-processing methods that might meet a low-cost criterion, the anodisation of the aluminium metal electrodes was deemed to be attractive since firstly it could resolve the observed corrosion problems of unmodified electrodes and secondly because aluminium oxide (alumina) has previously been established as biocompatible. Further, the anodisation could produce a porous electrode surface. Porous surfaces have been shown to produce enhanced cell-substrate adhesion, a factor important for correct function of cell-based biosensors. The research was therefore to investigate whether a biocompatible CMOS electrode could be formed by anodising the aluminium to form a porous alumina layer. Such an approach is believed to be novel, a claim substantiated by European Patent Office searches. Design variations and enhancements to be developed included improving the electrical characteristics of the electrode by modifying the insulating alumina barrier layer at the base of the pores. Proposed methods included electrochemical thinning of the oxide and deposition of a noble metal to reduce the barrier layer impedance.

Preliminary evaluations demonstrated for the first time the ability to anodise CMOS aluminium electrodes and allowed the electrical (e.g. anodising current density) and physical (such as pore pitch) attributes of the process to be characterised. Electrodes were shown to be biocompatible with a mammalian neuronal cell line (NG108-15). Cell vitality was found to be insensitive to surface chemistry and alumina pore pitch. Means by which to measure more directly cell-substrate adhesion were sought, but the established method using a parallel plate flow chamber was found to be inadequate. A novel centrifugation assay was therefore developed and this showed that neuronal cells adhere preferentially to porous alumina substrates with a large (206 nm) pore pitch versus smaller (17 nm, 69 nm) pitches or plain aluminium. Analysis of cell-substrate interfaces was performed by preparing TEM sections. Preliminary results indicated the cell protrudes into the large pores (206 nm pitch) but not small pores (69 nm pitch) and cells on aluminium substrates showed no discernable cleft. Both results warrant further investigation.

ICs for recording neuronal action potentials were designed using a commercial CMOS fabrication process. However, before ICs can be used as biosensors they must be assembled into packaging that can prevent the extracellular medium from creating electrical shorts to the IC bondwires. To avoid cumbersome methods reported in the literature and to achieve lifetimes suited to neuronal cell culture, two new prototype assembly methods were developed. Firstly, a method using a polyethylene glycol mould enabled improved manual
assembly and, secondly, a commercial partial encapsulation method (Quik-Pak Inc.) was evaluated. Biocompatibility of both methods was established through tests using the NG108-15 cell line. The Quik-Pak method was the preferred solution due to the high assembly yield, higher cell counts and fast throughput. Further work could be performed to establish the partial encapsulation method as the basis for commercial production of CMOS-based biosensors.

As expected, it was shown by electrochemical impedance spectroscopy (EIS) that without additional processing the high impedance of the porous alumina ($10^8 \Omega \cdot \text{cm}^2$ at $10^{-2}$ Hz) was not optimal for use as an electrode. A moderate reduction in impedance was achieved by electrochemical thinning of the barrier oxide but improved electrical characteristics ($10^2$–$10^4 \Omega \cdot \text{cm}^2$ at $10^{-2}$ Hz) were achievable by depositing platinum into the alumina pores. As a consequence of this work, new methods for real-time measurement of impedance were developed and helped gain new insights into the anodisation, electrochemical oxide thinning and electrodeposition processes. Enhancements to the process enabled the porosity of the alumina film to be modified for optimised cell adhesion by modification of the anodising electrolyte using polyethylene glycol. This technique had previously been achieved at 15ºC but was here modified to run at room temperature in order to meet the low-cost post-processing criterion.

Alumina pores are expected to have fairly uniform walls between the film’s surface and base. However, it is known that alloying aluminium with other elements disrupts this pore structure. Experiments showed that the small quantities of alloying elements in CMOS metallisation (1% silicon and 0.5% copper) are sufficient to disrupt the structure, with cross sections showing inter-linking pores. It was perceived that this could reduce the effectiveness of the porous alumina electrode in recording action potentials and therefore further development pursued a design variant incorporating a noble metal within the pores.

Electrodeposition into the porous alumina using platinum was found to be problematic due to difficulties in finding suitable plating bath operating conditions: operation at the higher temperatures used in industrial plating caused dissolution of the alumina and aluminium whereas lower temperatures caused poor plating and problems with gas evolution. It was also found that uniform plating of the 48 electrodes in the CMOS IC array was difficult to achieve. These problems were finally overcome through the use of gold deposition instead of platinum
and by plating CMOS pads using individual current sources. A final enhancement included the deposition of platinum black onto the gold surface to decrease further the electrode impedance (to $10^1$ $\Omega\cdot\text{cm}^2$ at $10^{-2}$ Hz) for improved signal-to-noise ratio during neuronal recordings.

A total of 26 CMOS ICs were successfully assembled and post-processed using anodisation, electrochemical barrier oxide thinning and uniform electrodeposition of gold and platinum black. These devices were tested using two applications. Firstly, neuronal cells (NG108-15 and primary rat neurons) were used to test the CMOS electrode array in the recording of action potentials. Secondly, an epithelial adenocarcinoma (Caco2) cell line was used to test the CMOS array as an impedance sensor (ECIS). Action potentials were successfully recorded using a rat dorsal root ganglion and the growth of a Caco2 monolayer was successfully measured using ECIS. Whilst the ability to record action potentials was proven, the success rate was poor. Further work is planned to understand the problems – probably related to the cell culture and cell plating protocols – since improved performance is required if the technology is to be commercialised. Other design variants may also be explored. For example, pores may be partially filled with gold and the alumina pore walls selectively etched back to leave a gold electrode with high surface area. This may allow the platinum black to be dispensed with. Alternatively, it may be possible to deposit platinum black within the alumina pores thereby avoiding an electrode surface above the plane of the IC passivation surface yet retaining a porous surface for enhanced adhesion and the platinum black for optimum impedance.

12.1 Future Work

It is acknowledged that this research is only a first step towards commercialisation and further work is needed to demonstrate the technology in a commercial context. Firstly, packaging will remain an issue: either new methods will be required (e.g. microtitre plates with ICs in each well) or the partial encapsulation process will need refining. Considerable effort will be required in terms of ‘design for manufacture’ as the CMOS IC must be capable of supporting the anodisation and electrodeposition processes and must integrate with other IC functional blocks such as amplifiers, data processing and communications circuits. Further still, once an IC design and post-processing steps are finalised, the manufacturability (yield, process parameters) and reliability will need optimising. Industry standard semiconductor reliability
tests can be adopted for this purpose, supplemented by additional tests specific to biosensors such as biocompatibility testing. The process must also be future proof against fast moving semiconductor technology and it will be necessary for the post-processing methods to periodically transition to newer CMOS fabrication processes. For example, further work is required to ensure that the anodisation and deposition processes will operate on the austriamicrosystems 0.35 µm process. This incorporates a 100 nm titanium nitride barrier layer instead of the 40 nm titanium metal layer used on the current set of ICs fabricated using their 0.8 µm process. From a commercial viewpoint, a more thorough understanding of projected production costs must be performed and a business risk assessment made against competing technologies. For example, bench top processing of ICs seems intrinsically cost effective, but scaling to production line volumes has yet to undergo rigorous business analysis. Also, if multiple ICs are to be integrated into the wells of microtitre plates, then acceptable production yield will need to be proven.

12.2 Closing Remarks

This research has met its overall objectives in developing a low-cost post-processing method for CMOS that complements the economies of scale of this ubiquitous IC technology. Adoption of the processes developed may foster the commercialisation of CMOS biosensors in drug discovery, neuroprosthetics, environmental applications and enable affordable research tools for bioscience.
References


[262] R. Egger, (hitkit@austriamicrosystems.com), 06 July 2007, austriamicrosystems AG, Email to AHD Graham (abmahdg@bath.ac.uk).


Appendix A  Electrophoretic D.C. Fields

Since cells have a net charge, it should be possible to use a d.c. field to manipulate them (i.e. d.c. electrophoresis). However, modelling a cell which is suspended in a fluid and where the cell is approaching a solid substrate is complex: factors to be considered are: double layers of fixed and motile ions form on the cell and on the planar charged surface; the double layers on the cell include an inner Stern layer and an outer diffuse layer; these charges create a Zeta potential which partially negate any net charge on the cell; the planar electrode surface charge can be similarly modelled most simply as an Inner Helmholtz Plane and Outer Helmholtz Plane, and in a more refined manner using the Gouy-Chapman model that accounts for thermal mixing of the outer layer to form a diffuse layer [97].

Galvanotropism is the ability to influence neuronal growth by application of extracellular electric fields (d.c. and pulsed). Papers in this field such as [383] also provide further insight into the more general case of cell-substrate adhesion. Other factors affecting the electrophoresis of cells include the glycocalyx and extracellular proteins that protrude from the cell membrane surface and may be polarised; the distribution of charge across the cell surface will also be non-uniform. Therefore, from the viewpoint of electrostatics, it is difficult to model the dynamics of biological cell manipulation toward a charged substrate. These issues are discussed further in [384].

The following experiment was devised to test whether cells could be manipulated using a d.c. field.

NG108-15 Electrode Bias Experiment

A passive CMOS MEA (‘WET3’) was plated with cells, the culture protocol as described in Section 5.3.1.1. Sterilisation of culture chamber and bath electrode (Ag/AgCl wire) was performed using ethanol. The 48 electrode pads were divided into four quadrants: two quadrants were controls with 0 V bias; the other two quadrants were each biased with positively and negatively. Biases of ±0.1 V (n = 3), ±0.2 V (n = 1) ±0.6 V (n = 1) and ±0.8 V (n = 1) were evaluated. Microscope inspection was used to determine whether there was any preferential adhesion of the stained cells to pads or passivation in any quadrant. The results
indicated that there was no effect: there was no discernible difference between the control and biased quadrants on any of the samples (Figure 139).

The experiment must be considered preliminary since the main body of the work showed that these unmodified CMOS pads readily corroded. The above results are therefore inconclusive. This experiment could be repeated as future work using the biocompatible electrodes developed (Chapter 9).

Figure 139. Cell distribution after ±0.6 V for 3 days. There is no discernable difference in number or location of cells in any quadrant, suggesting there was no effect of the d.c. bias on cell adhesion.
### Appendix B  Coverslip and IC Processing Reference

The table below summarises the experimental objectives and processing conditions for each set of coverslips and ICs. ‘Slip’ is an abbreviation of coverslip.

<table>
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<tr>
<th>Sample</th>
<th>Objective</th>
<th>Anodisation conditions</th>
<th>Electrodeposition conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-13</td>
<td>Preliminary methodology and barrier thinning expts</td>
<td>30 V, various thinning schemes</td>
<td>Pt CPA, 40-45°C, 50 mA pulsed; Pt P-Salt, 35°C, 50 mA, 15 mins</td>
<td>Solartron EIS capability proven. First set of EIS data. Basic Pt deposition capability demonstrated. Problems with corrosion in CPA.</td>
</tr>
<tr>
<td>14-17</td>
<td>Barrier thinning</td>
<td>30 V, various thinning schemes</td>
<td>Nickel: 35°C, +50 mA 8 ms / -3 V 2 ms, 500 ms period.</td>
<td>Poor metal deposition.</td>
</tr>
<tr>
<td>19-20</td>
<td>Agilent 4294A anodisation and EIS development</td>
<td>30 V, V-ramp thinning to 5 V</td>
<td>-</td>
<td>iBasic program capability; Anodisation through 4294A demonstrated with real-time EIS.</td>
</tr>
<tr>
<td>21</td>
<td>Pore widening with real-time EIS</td>
<td>30 V, 1/3 thickness, Vramp to 5 V, then passive etch</td>
<td>-</td>
<td>Process understood. Corroboration with other pore-widening etch rates.</td>
</tr>
<tr>
<td>22-23</td>
<td>Effects of Si and Cu alloying elements</td>
<td>30 V, 1/3 thickness, Vramp to 5 V.</td>
<td>-</td>
<td>Reduced</td>
</tr>
<tr>
<td>25</td>
<td>Real-time EIS for electrodeposition</td>
<td>30 V, complete, to Isteady/10</td>
<td>Nickel: 35°C, +70 mA 8 ms / -3 V 2 ms, 500 ms period.</td>
<td></td>
</tr>
<tr>
<td>27-39</td>
<td>Reverse bias preliminary tests</td>
<td>30 V, various thinning schemes; Rev bias pulses</td>
<td>Nickel: 35°C, +70 mA 8 ms / -3 V 2 ms, 500 ms period.</td>
<td></td>
</tr>
<tr>
<td>47-50</td>
<td>PEG pore wall thickness modulation</td>
<td>15-30% PEG, 2% phos, 21°C</td>
<td>Nickel: 35°C, +70 mA 8 ms / -3 V 2 ms.</td>
<td>No effect on porosity</td>
</tr>
<tr>
<td>51-59</td>
<td>PEG pore wall thickness modulation</td>
<td>0-80% PEG, 15°C, oxalic, double-anodised films</td>
<td>Nickel: 35°C, +70 mA 8 ms / -3 V 2 ms.</td>
<td>Literature reproduced</td>
</tr>
<tr>
<td>60-63</td>
<td>PEG pore wall thickness modulation</td>
<td>40% PEG, 0.5%-4% phosphoric, 21°C</td>
<td>Nickel: 35°C, +70 mA 8 ms / -3 V 2 ms.</td>
<td>Porosity manipulation achieved at 21°C.</td>
</tr>
<tr>
<td>66-69</td>
<td>Pt deposition and pulse duty cycle</td>
<td>39 V, 40% PEG, 1% phosphoric, 21°C</td>
<td>Pt P-salt: 35°C, +70 mA 8 ms / -3 V 2 ms, 8%-50% duty</td>
<td>Poor platinum coverage. Improved</td>
</tr>
<tr>
<td>70-77</td>
<td>P-salt bath temperature and duration</td>
<td>39 V, 40% PEG, 1% phosphoric, 21°C</td>
<td>35-65°C; 60-180 mins; VSET=5 V; 9.9 V; P-salt, CPA.</td>
<td>Improved Pt coverage with 120-180 mins; etching at higher P-salt temperatures. 9.9 V and CPA result poor.</td>
</tr>
<tr>
<td>79-89</td>
<td>Alternate plating schemes.</td>
<td>60 V, 4% phosphoric, 21°C</td>
<td>Constant I (15 mA cm⁻²) w/ P-salt, CPA pH 7, CPA pH 4; pulsed 2-5 V (+15-20 mA) / -2 V P-salt, CPA pH 7;</td>
<td>No clear improvements in Pt plating quality.</td>
</tr>
</tbody>
</table>

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83 Coverslip serial numbers unless stated as IC
84 ‘complete’ means anodised to the base of the Al layer. ‘1/3 thickness’ is the same as ‘partial anodisation’. ‘Isteady’ is the steady state porous layer anodising current. Vramp is a voltage-controlled ramp.
<table>
<thead>
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<th>IC</th>
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<th>Plating scheme, Pt-black</th>
<th>Second lot for cell culture experiments</th>
<th>Third lot for cell culture experiments</th>
<th>Final specimen for SEM/FIB</th>
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<tr>
<td>IC1</td>
<td>-</td>
<td>30 V, V-ramp to 5 V</td>
<td>39 V, 1% phos + 40% PEG, thin to 5 V, Pt P-salt: 35°C, 5 V 8 ms / - 3 V 2 ms, 8%-50% duty</td>
<td>60 V, 4% phosphoric, to cusp, V-ramp</td>
<td>60 V, V-ramp to 5 V</td>
<td>60 V, V-ramp to 5 V</td>
<td>60 V, V-ramp to 5 V</td>
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<td>60 V, V-ramp to 5 V</td>
<td>60 V, V-ramp to 5 V</td>
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<tr>
<td>IC2</td>
<td>Initial IC anodisation and plating</td>
<td>-</td>
<td>-</td>
<td>Uniform anodisation achieved. Poor control of plating.</td>
<td>60 V, partial or complete anodisation, V-ramp to 5 V</td>
<td>-</td>
<td>-</td>
<td>Pt P-salt: 35°C, 5 V 8 ms / - 3 V 2 ms, 8%-50% duty</td>
<td>Pt P-salt: 35°C, 5 V 8 ms / - 3 V 2 ms, 8%-50% duty</td>
<td>Pt P-salt: 35°C, 5 V 8 ms / - 3 V 2 ms, 8%-50% duty</td>
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<tr>
<td>IC3</td>
<td>-</td>
<td>60 V, V-ramp to 5 V</td>
<td>60 V, 4% phosphoric, 21°C, Pt P-salt: 35°C, 5 V 8 ms / - 3 V 2 ms, 8%-50% duty</td>
<td>Initial lot for NG108-15 cell culture experiments. Continued use after reproprocessing.</td>
<td>60 V, V-ramp to 5 V</td>
<td>60 V, V-ramp to 5 V</td>
<td>60 V, V-ramp to 5 V</td>
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<td>60 V, V-ramp to 5 V</td>
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<td>IC4</td>
<td>-</td>
<td>12 V, a.c. pulsed 2-5 V (+15-20 mA) / -2 V, P-salt, CPA pH 7 , ramping bias</td>
<td>-</td>
<td>12 V, a.c. pulsed 2-5 V (+15-20 mA) / -2 V, P-salt, CPA pH 7 , ramping bias</td>
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<td>IC5</td>
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<td>Pt P-salt: 35°C, 5 V 8 ms / - 3 V 2 ms, 8%-50% duty</td>
<td>Poor or no plating</td>
<td>No effect of thinning scheme on plating quality</td>
<td>Poor or no plating</td>
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<tr>
<td>IC6</td>
<td>-</td>
<td>Initial IC anodisation and plating</td>
<td>Pt P-salt: 35°C, 5 V 8 ms / - 3 V 2 ms, 8%-50% duty, Syringe agitation.</td>
<td>Initial IC anodisation and plating</td>
<td>Pt P-salt: 35°C, 5 V 8 ms / - 3 V 2 ms, 8%-50% duty</td>
<td>Pt P-salt: 35°C, 5 V 8 ms / - 3 V 2 ms, 8%-50% duty</td>
<td>Pt P-salt: 35°C, 5 V 8 ms / - 3 V 2 ms, 8%-50% duty</td>
<td>Pt P-salt: 35°C, 5 V 8 ms / - 3 V 2 ms, 8%-50% duty</td>
<td>Pt P-salt: 35°C, 5 V 8 ms / - 3 V 2 ms, 8%-50% duty</td>
<td>Pt P-salt: 35°C, 5 V 8 ms / - 3 V 2 ms, 8%-50% duty</td>
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<tr>
<td>IC7</td>
<td>-</td>
<td>60 V, 4% phosphoric, 21°C, SCE ref electrode, Extended V-ramp thinning</td>
<td>60 V, 4% phosphoric, 21°C, SCE ref electrode, Extended V-ramp thinning</td>
<td>60 V, 4% phosphoric, 21°C, SCE ref electrode, Extended V-ramp thinning</td>
<td>60 V, 4% phosphoric, 21°C, SCE ref electrode, Extended V-ramp thinning</td>
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<td>40-60 mA cm^-2</td>
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**Notes:**
- **IC** represents Initial IC anodisation and plating.
- **CV** represents Constant Voltage.
- **SEM/FIB** represents Scanning Electron Microscopy/Focused Ion Beam.
- **P-Stat** represents Potentiostat.
Appendix C  iBasic Anodisation Impedance Script for 4294A

1 ! ******************************************
2 ! Manual stop anodisation; single thinning;
3 ! Constant 5 V anodisation
4 ! ******************************************
5 DIM Mon_osc_v(1:11),Mon_osc_i(1:11),Mon_bias(1:11),Swp_prm(1:11)
6 DIM Trca(1:1,1:2),Trcb(1:1,1:2),Trcc(1:1,1:2)
7 DIM
8 Osc_mode$[9],Mon_bias$[9],Header$[9],Buff$[9],Img$[72],Img2$[72],Wrt$[90]
9 DIM Results(1:60,1:7),File$[12]
10 INTEGER Num_res,L,Again,Flag
11 REAL Osc_pow,Dc_bias,Delay,V_rate,T_on,T_start,T_stop,Delta_t,V1,V2,Vt
12 CLEAR SCREEN
13 IF SYSTEM$("SYSTEM ID")="HP4294A" THEN
14   ASSIGN @Hp4294A TO 800
15 ELSE
16   ASSIGN @Hp4294A TO 717
17 END IF
18!
19 T_on=0
20 Again=1
21 Osc_mode$="VOLT"
22 Osc_pow=.2
23 Dc_b_rng$="M100"
24 Nop_cycles=11.
27 ! Img$ and Img2$ will create a runtime error if exp exceeds 27 hours
28 FOR I=1 TO Nop_cycles
29   Mon_bias(I)=0         ! Write zeros into array
30 NEXT I
31 !
32 OUTPUT @Hp4294A;";DISA HIHB"
33 OUTPUT @Hp4294A;"PRES"
34 OUTPUT @Hp4294A;"STAR 40 HZ"
35 OUTPUT @Hp4294A;"STOP 1 MHZ"
36 OUTPUT @Hp4294A;"POIN ";Nop_cycles
37 OUTPUT @Hp4294A;"POWMOD ";&Osc_mode$
38 OUTPUT @Hp4294A;"POWE ";Osc_pow
39 OUTPUT @Hp4294A;"DCMOD VOLT"
40 Dc_bias=0.
41 OUTPUT @Hp4294A;"DCV ";Dc_bias
42 OUTPUT @Hp4294A;"DCRNG ";&Dc_b_rng$
43 ! Leave DC OFF to measure DUT at open circuit potential (OCP)
44 OUTPUT @Hp4294A;"OMON ON"
45 OUTPUT @Hp4294A;"BMON CURR"
46 OUTPUT @Hp4294A;"SWPT LOG"
47!
48 ! Request user inputs data file name
49 CALL Inp_file_name(File$)
50 ! Open file for writing results
51 CREATE File$".TXT",5
ASSIGN @Path_1 TO File$".TXT"; FORMAT ON

! **************************************
! MAIN PROGRAM START HERE ************
! Pause until soft key is pressed
OUTPUT @Hp4294A;"USKEY"

Loop0:  !
Start_time=TIMEDATE
ON KEY 8 LABEL "START TEST" GOTO Loop1
WAIT .5
GOTO Loop0
! Loop until soft key is pressed
Loop1:  ! Measure before anodisation (using open circuit potential)
Delay=5 ! readout every 5 s
Flag=1 ! Do not turn on DC bias for this test
GOSUB Bias_dut
GOSUB Measure_dut
OUTPUT @Hp4294A;"USKEY"
ON KEY 8 LABEL "START BIAS" GOTO Next_test2
GOTO Loop1

Next_test2:  ! Start anodisation
Dc_bias=30.
OUTPUT @Hp4294A;"DCV ";Dc_bias
Delay=15 ! readout every 15 s
Loop2:  !
GOSUB Bias_dut
GOSUB Measure_dut
OUTPUT @Hp4294A;"USKEY"
ON KEY 7 LABEL "V RAMP THINNING" GOTO Next_test3
ON KEY 8 LABEL "END EXPT" GOTO End_sequence
GOTO Loop2

Next_test3:  ! Voltage Ramp Thinning
Delay=10 ! readout every 10 s
T1=T_on ! set ramp start time to current on-time
Delta_t=60*5 ! thinning 'on' time is 5 mins
V1=30
V2=5
V_rate=(V1-V2)/Delta_t
Loop3:  !
Vt=V1-V_rate*(T_on-T1) ! present V decreases with on-time
Dc_bias=Vt
OUTPUT @Hp4294A;"DCV ";Dc_bias
GOSUB Bias_dut
GOSUB Measure_dut
ON KEY 7 LABEL "GOTO 5 V CONSTANT" GOTO Next_test4
ON KEY 8 LABEL "END EXPT" GOTO End_sequence
IF Vt<V2 THEN GOTO Next_test4
GOTO Loop3

Next_test4:  ! Start 5 V constant thinning
Delay=30 ! readout every 30 s
Dc_bias=5.
Delta_t=60*5 ! constant-V time is 5 mins
T1=T_on ! set start time to present on-time
OUTPUT @Hp4294A;"DCV ";Dc_bias
Loop4:  !
GOSUB Bias_dut
GOSUB Measure_dut
OUTPUT @Hp4294A;"USKEY"
ON KEY 7 LABEL " " GOTO End_sequence  ! dummy
ON KEY 8 LABEL "END EXPT" GOTO End_sequence
IF T_on>(T1+Delta_t) THEN GOTO End_sequence
GOTO Loop4
!
GOTO End_sequence
!
! GENERAL PURPOSE BIAS ROUTINE
!
Bias_dut:  
T_start=TIMEDATE
IF Flag=0 THEN OUTPUT @Hp4294A;"DCO ON"
Flag=0    ! reset flag for next test
WAIT Delay    ! Bias for a pre-defined period
OUTPUT @Hp4294A;"SING"  ! Sweep is needed to measure IDC
OUTPUT @Hp4294A;"*OPC?"    ! Check for ready status
ENTER @Hp4294A;Buff$  
OUTPUT @Hp4294A;"OUTPDC?"    ! Measure DC current during bias
ENTER @Hp4294A USING ",","A";Header$
ENTER @Binary;Mon_bias(*)
OUTPUT @Hp4294A;"DCO OFF"
T_stop=TIMEDATE
T_on=T_on+(T_stop-T_start)       ! Add time biased onto t_on
RETURN
!
!
! MEASUREMENT SUBROUTINE ***********
!
Measure_dut:  
OUTPUT @Hp4294A;"MEAS IMPH"
OUTPUT @Hp4294A;"SING"
OUTPUT @Hp4294A;"*OPC?"
ENTER @Hp4294A;Buff$
OUTPUT @Hp4294A;"AUTO"
!
OUTPUT @Hp4294A;"FORM3"
!
OUTPUT @Hp4294A;"OUTPDC?"
ENTER @Hp4294A USING ",","A";Header$
ENTER @Binary;Mon_bias(*)
OUTPUT @Hp4294A;"AUTO"
!
OUTPUT @Hp4294A;"OUTPSWPRM?"
ENTER @Hp4294A USING ",","A";Header$
ENTER @Binary;Swp_prm(*)
OUTPUT @Hp4294A;"AUTO"
!
Transfer data trace A array into a binary array
OUTPUT @Hp4294A;"TRAC A"
OUTPUT @Hp4294A;"OUTPDTRC?"
ENTER @Hp4294A USING ",","A";Header$
ENTER @Binary;Trca(*)
OUTPUT @Hp4294A;"AUTO"
!
Transfer data trace B array into a binary array
OUTPUT @Hp4294A;"TRAC B"
OUTPUT @Hp4294A;"OUTPDTRC?"
ENTER @Hp4294A USING ",","A";Header$
ENTER @Binary;Trcb(*)
OUTPUT @Hp4294A;"AUTO"
!
Change Trace A to display real-imag and store data
OUTPUT @Hp4294A;"TRAC A"
OUTPUT @Hp4294A;"MEAS COMP"
OUTPUT @Hp4294A;"AUTO"
1690 OUTPUT @Hp4294A; "OUTPDTRC?"
1700 ENTER @Hp4294A USING ",,8 A"; Header$
1710 ENTER @Binary;Trcc(*)
1720 ENTER @Hp4294A USING ",,1 A"; Buff$
1730 !
1740 ! Output measurement results to screen and file
1750 !
1760 IF Num_res=1 THEN ! Write headers if first iteration
1770 PRINT "DC CURRENT AND IMPEDANCE DATA"
1780 PRINT "Elapsed time[s] DC_Measured[A or V] F[Hz] |Z|[ohm] theta[deg]"
1790 OUTPUT @Path_1; "Elapsed_time[s] DC_applied[V_or_A] DC_measured[V_or_A] F[Hz] |Z|[ohm] theta[deg] Z-real[ohm] Z-imag[ohm]"
1800 END IF
1810 FOR I=1 TO Nop_cycles ! Write a record for each of the sweep points
1820 PRINT USING Img$; T_on, Dc_bias, Mon_bias(I), Swp_prm(I), Trca(I,1), Trcb(I,1)
1830 ! Write results to the open file
1840 OUTPUT Wrt$ USING Img2$; T_on, Dc_bias, Mon_bias(I), Swp_prm(I), Trca(I,1), Trcb(I,1), Trcc(I,1), Trcc(I,2)
1850 OUTPUT @Path_1; Wrt$
1860 NEXT I
1870 Num_res=Num_res+1
1880 RETURN
1890 !
1900 End_sequence: !
1910 !
1920 ! Close the results file
1930 ASSIGN @Path_1 TO *
1940 ! WAIT 4
1950 ! backup the results file - caution - Agilent bugs
1960 ! Wrt$="FILC ***&File$&.TXT"",""DISK"",****&File$&.BAK"",""FLASH"
1970 ! PRINT Wrt$
1980 ! OUTPUT @Hp4294A; Wrt$
1990 !
2000 ! Power Down the DC O/P at the very end
2010 !
2020 OUTPUT @Hp4294A; "DCO OFF"
2030 END
2040 !
2050 ! **********************************************
2060 ! I/O SUBROUTINES **********************************************
2070 ! **********************************************
2080 ! File Name Input Function
2090 !
2100 SUB Inp_file_name(Inp_name$)
2110 DIM Inp_char$[30]
2120 ON ERROR GOTO Inp_start
2130 Inp_start: !
2140 PRINT "Input Results File Name (without extension)"
2150 INPUT "File Name? ", Inp_char$
2160 Inp_name$=UPC$(Inp_char$)
2170 IF LEN(Inp_name$)>8 THEN Inp_start
2180 PRINT "Input Name: ", Inp_name$
2190 INPUT "OK? [Y/N]", Inp_char$
2200 IF UPC$(Inp_char$)<"Y" THEN Inp_start
2210 OFF ERROR
2220 SUBEND
Appendix D  iBasic Deposition Impedance Script for 4294A

10 ! **********************************************
20 ! MEASURES IMPEDANCE DURING ELECTRODEPOSITION
30 ! Requires GPIB between 4294A and Agilent 33220A
40 ! Requires bit 0 of 8-bit O/P connected to relay
50 ! Requires Ext. bias adaptor as 4294A fixture
60 ! Requires DEPOSIT4.STA compensation file
70 ! **********************************************
80 !
90 DIM Mon_osc_v(1:11),Mon_osc_i(1:11),Mon_bias(1:11),Swp_prm(1:11)
100 DIM Trca(1:11 ,1:2),Trcb(1:11 ,1:2),Trcc(1:11 ,1:2)
110 DIM Osc_mode$[9],Mon_bias$[9],Header$[9],Buff$[9],Img$[72],Img2$[72],Wrt$[90]
120 DIM Results(1:60 ,1:7),File$[12]
130 INTEGER Num_res,L,Flag
140 REAL Osc_pow,Dc_bias,Delay,V_rate,T_on,T_start,T_stop,Delta_t,V1 ,V2 ,Vt
150 CLEAR SCREEN
160 IF SYSTEM$("SYSTEM ID")="HP4294A" THEN
170 ASSIGN @Hp4294A TO 800
180 ASSIGN @Binary TO 800;FORMAT OFF
190 ELSE
200 ASSIGN @Hp4294A TO 717
210 ASSIGN @Binary TO 717;FORMAT OFF
220 END IF
230 ! Set up comms to Agilent 33220A Waveform generator
240 ASSIGN @Wave TO 710
250 ! ASSIGN @Binary TO 710;FORMAT OFF
260 !
270 T_on=0
280 Num_res=1
290 Osc_mode$="VOLT"
300 Osc_pow=.2
310 Dc_b_rng$="M100"
320 Nop_cycles=11.
350 ! Img$ and Img2$ will create a runtime error if expt exceeds 27 hours
360 !
370 OUTPUT @Hp4294A;"RECD DEPOSIT1.STA"
380 OUTPUT @Hp4294A;"BWFFACT 3"
390 OUTPUT @Hp4294A;"FMT LOGY"
400 OUTPUT @Hp4294A;"DISA HIHB"
410 OUTPUT @Hp4294A;"PRES"
420 OUTPUT @Hp4294A;"STAR 40 HZ"
430 OUTPUT @Hp4294A;"STOP 1 MHZ"
440 OUTPUT @Hp4294A;"POIN ";Nop_cycles
450 OUTPUT @Hp4294A;"POWMOD ";Osc_mode$
460 OUTPUT @Hp4294A;"POWE ";Osc_pow
470 OUTPUT @Hp4294A;"DCMOD VOLT"
480 Dc_bias=0.
490 OUTPUT @Hp4294A;"DCV ";Dc_bias
500 OUTPUT @Hp4294A;"DCRNG ";Dc_b_rng$
510 OUTPUT @Hp4294A:"OMON ON"
520 OUTPUT @Hp4294A:"BMON CURR"
530 OUTPUT @Hp4294A:"SWPT LOG"
540 !
550 ! Request user inputs data file name
560 CALL Inp_file_name(File$)
570 ! Open file for writing results
580 CREATE File$&".TXT",5
590 ASSIGN @Path_1 TO File$&".TXT";FORMAT ON
600 !
610 !
620 ! ************************************************************************
630 ! MAIN PROGRAM START HERE **********
640 ! Pause until soft key is pressed
650 OUTPUT @Hp4294A:"USKEY"
660 Loop0: !
670 Start_time=TIMEDATE
680 ON KEY 8 LABEL "START TEST" GOTO Next_test5
690 WAIT .1
700 GOTO Loop0
710 ! Loop until soft key is pressed
720 ! ON ERROR GOSUB Power_down ! Turn off EIS relay if error
730 Next_test5: ! Start pore-widening monitoring (OCP)
740 OUTPUT @Hp4294A:"USKEY"
750 ON KEY 1 LABEL "15 s DELAY" GOSUB Delay_15
760 ON KEY 2 LABEL "30 s DELAY" GOSUB Delay_30
770 ON KEY 3 LABEL "1 min DELAY" GOSUB Delay_60
780 ON KEY 4 LABEL "2 min DELAY" GOSUB Delay_120
790 ON KEY 5 LABEL "5 min DELAY" GOSUB Delay_300
800 ON KEY 8 LABEL "END EXPT" GOTO End_sequence
810 Delay=10 ! readout every 60 s
820 Dc_bias=0.
830 OUTPUT @Hp4294A:"DCV ";Dc_bias
840 Loop4: !
850 OUTPUT @Wave;"OUTP OFF" ! turn off plating
860 WAIT .2 ! Let V settle before switching
870 OUTPUT @Hp4294A;"OUT8 IO 1" ! Turn on TTL O/P to set relay
880 WAIT .5 ! Let settle to OCP
890 GOSUB Measure_dut
900 OUTPUT @Hp4294A;"OUT8 IO 0" ! Turn off TTL O/P to reset relay
910 OUTPUT @Wave;"OUTP ON"
920 WAIT Delay ! delay before next readout
930 GOTO Loop4
940 !
950 Delay_15: !
960 Delay=15
970 RETURN
980 Delay_30: !
990 Delay=30
1000 RETURN
1010 Delay_60: !
1020 Delay=60
1030 RETURN
1040 Delay_120: !
1050 Delay=120
1060 RETURN
1070 Delay_300: !
1080 Delay=300
1090 RETURN
1100 GOTO End_sequence
1110 !
! MEASUREMENT SUBROUTINE ***********
Measure_dut:  !
OUTPUT @Hp4294A;"MEAS IMPH"
OUTPUT @Hp4294A;"SING"
OUTPUT @Hp4294A;"*OPC?"
ENTER @Hp4294A;Buff$
OUTPUT @Hp4294A;"AUTO"
!
OUTPUT @Hp4294A;"FORM3"
!
OUTPUT @Hp4294A;"OUTPDC?"
ENTER @Hp4294A USING ",",8 A$;Header$
ENTER @Binary;Mon_bias(*)
ENTER @Hp4294A USING ",",1 A$;Buff$
!
OUTPUT @Hp4294A;"OUTPSWPRM?"
ENTER @Hp4294A USING ",",8 A$;Header$
ENTER @Binary;Swp_prm(*)
ENTER @Hp4294A USING ",",1 A$;Buff$
!
Transfer data trace A array into a binary array
OUTPUT @Hp4294A;"TRAC A"
OUTPUT @Hp4294A;"OUTPDTRC?"
ENTER @Hp4294A USING ",",8 A$;Header$
ENTER @Binary;Trca(*)
ENTER @Hp4294A USING ",",1 A$;Buff$
!
Transfer data trace B array into a binary array
OUTPUT @Hp4294A;"TRAC B"
OUTPUT @Hp4294A;"OUTPDTRC?"
ENTER @Hp4294A USING ",",8 A$;Header$
ENTER @Binary;Trcb(*)
ENTER @Hp4294A USING ",",1 A$;Buff$
!
Change Trace A to display real-imag and store data
OUTPUT @Hp4294A;"TRAC A"
OUTPUT @Hp4294A;"MEAS COMP"
OUTPUT @Hp4294A;"AUTO"
OUTPUT @Hp4294A;"OUTPDTRC?"
ENTER @Hp4294A USING ",",8 A$;Header$
ENTER @Binary;Trcc(*)
ENTER @Hp4294A USING ",",1 A$;Buff$
!
Output measurement results to screen and file
!
T_on=TIMEDATE-Start_time
IF Num_res=1 THEN        ! Write headers if first iteration
PRINT "DC CURRENT AND IMPEDANCE DATA"
PRINT "Elaspsed time[s] DC_Measured[A or V] F[Hz] |Z|[ohm] theta[deg]"
OUTPUT @Path_1;"Elaspsed_time[s] DC_applied[V_or_A] DC_measured[V_or_A]  F[Hz]  |Z|[ohm] theta[deg]  Z-real[ohm]  Z-imag[ohm]"
END IF
FOR I=1 TO Nop_cycles    ! Write a record for each of the sweep points
PRINT USING Img$;T_on,Dc_bias,0 ,Swp_prm(I),Trca(I,1),Trcb(I,1)
WRITE results to the open file
OUTPUT Wrt$ USING
Img2$;T_on,Dc_bias,0 ,Swp_prm(I),Trca(I,1),Trcb(I,1),Trcc(I,1),Trcc(I,2)
END IF
NEXT I
1690   Num_res=Num_res+1
1700   RETURN
1710   !
1720   Power_down:  !
1730   OUTPUT @Hp4294A;"OUT8 IO 0"  ! Turn off TTL O/P to reset relay
1740   OUTPUT @Wave;"OUTP OFF"  ! Turn off waveform generator
1750   RETURN
1760   !
1770   End_sequence:  !
1780   !
1790   ! Close the results file
1800   ASSIGN @Path_1 TO *
1810   ! WAIT 4
1820   ! backup the results file - caution - Agilent bugs
1830   ! Wrt$="FILC "&Inp_name$&".TXT","DISK",""&Inp_name$&".BAK",""FLASH"
1840   ! PRINT Wrt$
1850   ! OUTPUT @Hp4294A;Wrt$
1860   !
1870   ! Power Down the DC O/P at the very end
1880   !
1890   OUTPUT @Hp4294A;"DCO OFF"
1895   OUTPUT @Wave;"OUTP OFF"  ! Turn off waveform generator
1900   OUTPUT @Hp4294A;"OUT8 IO 0"  ! Check TTL O/P is off to reset relay
1910   END
1920   !
1930   ! **********************************************
1940   ! I/O SUBROUTINES ****************************
1950   ! **********************************************
1960   ! File Name Input Function
1970   !
1980   SUB Inp_file_n
1990   Subname(Inp_name$)
2000   DIM Inp_char$[30]
2010   ON ERROR GOTO Inp_start
2020   Inp_start:  !
2030   PRINT "Input Results File Name (without extension)"
2040   INPUT "File Name? ",Inp_char$
2050   Inp_name$=UPC$(Inp_char$)
2060   IF LEN(Inp_name$)>8 THEN Inp_start
2070   PRINT "Input Name: ";Inp_name$
2080   INPUT "OK? [Y/N]",Inp_char$
2090   IF UPC$(Inp_char$)<"Y" THEN Inp_start
2100   OFF ERROR
2110   SUBEND
2120   !
Appendix E  iBasic Platinum Black Deposition Script for 4294A

10  ! ******************************************************
20  ! IC PT-BLACK DEPOSITION WITH |Z| MEAS
30  ! 50 V APPLIED THROUGH 68 Mohm RESISTORS
40  ! Requires GPIB to Keithley SourceMeter
50  ! Requires bit 0 of 8-bit O/P connected to relay
60  ! Requires compensation file
70  ! ******************************************************
80  !
90  DIM Mon_osc_v(1:11),Mon_osc_i(1:11),Mon_bias(1:11),Swp_prm(1:11)
100 DIM Trca(1:11 ,1:2),Trcb(1:11 ,1:2),Trcc(1:11 ,1:2)
110 DIM Osc_mode$[9],Mon_bias$[9],Header$[9],Buff$[9],Img$[72],Img2$[72],Wrt$[90]
120 DIM Results(1:60 ,1:7),File$[12]
130 INTEGER Num_res,L,Flag
140 REAL Osc_pow,Dc_bias,Delay,V_rate,T_on,T_stop,Delta_t,V,V2,Vt
150 CLEAR SCREEN
160 IF SYSTEM$("SYSTEM ID")="HP4294A" THEN
170  ASSIGN @Hp4294A TO 800
180  ASSIGN @Binary TO 800;FORMAT OFF
190 ELSE
200  ASSIGN @Hp4294A TO 717
210  ASSIGN @Binary TO 717;FORMAT OFF
220 END IF
230 !
240 T_on=0
250 Num_res=1
260 Osc_mode$="VOLT"
270 Osc_pow=.2
280 Dc_b_rng$="M100"
290 Nop_cycles=11.
300 Img$="DDDDD.DD,X,MD.2 DE,X,MD.3 DE,X,D.2 DE,X,D.3 DE,X,MDDD.DD"
320 ! Img$ and Img2$ will create a runtime error if expt exceeds 27 hours
330 !
340 GCLEAR  ! Create a I versus t graph behind the instrument screen
350 ! Draw lines to divide X-axis
360 FOR I=0 TO 10
370 MOVE (590/10)*I,205
380 DRAW (590/10)*I,215
390 NEXT I
400 MOVE 0 ,210
410 DRAW 590 ,210
420 ASSIGN @Meter TO 710  ! set up the SourceMeter
430 OUTPUT @Meter;"*RST"
440 ! OUTPUT @Meter;"*OPC"  ! use status
450 OUTPUT @Meter;"SOUR:FUNC VOLT"
460 OUTPUT @Meter;"SOUR:VOLT:MODE FIX"
470 OUTPUT @Meter;"SOUR:VOLT:RANG 100"
480 OUTPUT @Meter;"SOUR:VOLT:LEV 0"
490 OUTPUT @Meter;"SENS:FUNC ""VOLT"""
Dc_bias=50.  ! SET 50 V across 68 Mohm.

OUTPUT @Hp4294A;";BWFACT 3"
OUTPUT @Hp4294A;";FMT LOGY"
OUTPUT @Hp4294A;";DISA HIHB"
OUTPUT @Hp4294A;";PRES"
OUTPUT @Hp4294A;";STAR 40 HZ"
OUTPUT @Hp4294A;";STOP 1 MHZ"
OUTPUT @Hp4294A;";POIN ";Nop_cycles
OUTPUT @Hp4294A;";POWMOD ";Osc_mode$
OUTPUT @Hp4294A;";POWE ";Osc_pow
OUTPUT @Hp4294A;";DCMOD VOLT"
OUTPUT @Hp4294A;";DCV ";0
OUTPUT @Hp4294A;";DCRNG ";Dc_b_rng$
OUTPUT @Hp4294A;";OMON ON"
OUTPUT @Hp4294A;";BMON CURR"
OUTPUT @Hp4294A;";SWPT LOG"

! Request user inputs data file name
CALL Inp_file_name(File$)

CREATE File$&".TXT",5
ASSIGN @Path_1 TO File$&".TXT";FORMAT ON

! MAIN PROGRAM START HERE ************
! Pause until soft key is pressed
OUTPUT @Hp4294A;"USKEY"

LOOP0:  !
ON KEY 8 LABEL "START TEST" GOTO Loop1
WAIT .1
GOTO Loop0

! Loop until soft key is pressed
!
LOOP1:  ! Measure before plating (at open circuit potential)
Start_time=TIMEDATE
OUTPUT @Hp4294A;"OUT8 IO 1"  ! Turn on TTL O/P to set relay
OUTPUT @Hp4294A;"USKEY"
ON KEY 8 LABEL "START PLATING" GOTO Next_test5
LOOP1 a:  !
WAIT .5  ! Let settle to OCP
GOSUB Measure_dut
Delay=5 ! readout every 5 s
GOTO Loop1 a

! 1000 Next_test5:  ! Start plating
OUTPUT @Hp4294A;"USKEY"
ON KEY 1 LABEL "5 s DELAY" GOSUB Delay_5
ON KEY 2 LABEL "10 s DELAY" GOSUB Delay_10
ON KEY 3 LABEL "15 s DELAY" GOSUB Delay_15
ON KEY 4 LABEL "PAUSE" GOTO Paused
ON KEY 8 LABEL "END EXPT" GOTO End_sequence
Delay=10 ! default readout is every 10 s
OUTPUT @Hp4294A;"DCV ";0
LOOP4:  !
OUTPUT @Meter;";SOUR:VOLT:LEV 0"  ! turn off plating
1110 WAIT .2 ! Let V settle before switching
1120 OUTPUT @Hp4294A;"OUT8 IO 1" ! Turn on TTL O/P to set relay
1130 WAIT .5 ! Let settle to OCP
1140 GOSUB Measure_dut
1150 OUTPUT @Hp4294A;"OUT8 IO 0" ! Turn off TTL O/P to reset relay
1160 OUTPUT @Meter;";SOUR:VOLT:LEV ";Dc_bias
1170 WAIT Delay ! delay before next readout
1180 GOTO Loop4
1190!
1200 Delay_5: !
1210 Delay=5
1220 RETURN
1230 Delay_10: !
1240 Delay=10
1250 RETURN
1260 Delay_15: !
1270 Delay=15
1280 RETURN
1290 Paused: !
1300 OUTPUT @Meter;";SOUR:VOLT:LEV 0"
1310 Pause_loop: !
1320 ON KEY 4 LABEL "RESUME" GOTO Next_test5
1330 WAIT .5
1340 GOTO Pause_loop
1350!
1360 GOTO End_sequence
1370!
1380 ! MEASUREMENT SUBROUTINE ************
1390 Measure_dut: !
1400 OUTPUT @Hp4294A;"MEAS IMPH"
1410 OUTPUT @Hp4294A;"SING"
1420 OUTPUT @Hp4294A;"*OPC?"
1430 ENTER @Hp4294A;Buff$
1440 OUTPUT @Hp4294A;"AUTO"
1450!
1460 OUTPUT @Hp4294A;"FORM3"
1470!
1480 ! OUTPUT @Hp4294A;"OUTPDC?"
1490 ! ENTER @Hp4294A USING ",8 A";Header$!
1500 ! ENTER @Binary;Mon_bias(*)
1510 ! ENTER @Hp4294A USING ",1 A";Buff$
1520!
1530 OUTPUT @Hp4294A;"OUTPSWPRM?"
1540 ENTER @Hp4294A USING ",8 A";Header$
1550 ENTER @Binary;Swp_prm(*)
1560 ENTER @Hp4294A USING ",1 A";Buff$
1570!
1580 ! Transfer data trace A array into a binary array
1590 OUTPUT @Hp4294A;"TRAC A"
1600 OUTPUT @Hp4294A;"OUTPDTRC?"
1610 ENTER @Hp4294A USING ",8 A";Header$
1620 ENTER @Binary;Trca(*)
1630 ENTER @Hp4294A USING ",1 A";Buff$
1640!
1650 ! Transfer data trace B array into a binary array
1660 OUTPUT @Hp4294A;"TRAC B"
1670 OUTPUT @Hp4294A;"OUTPDTRC?"
1680 ENTER @Hp4294A USING ",8 A";Header$
1690 ENTER @Binary;Trcb(*)
1700 ENTER @Hp4294A USING ",1 A";Buff$
1710!
1720    ! Change Trace A to display real-imag and store data
1730    OUTPUT @Hp4294A;"TRAC A"
1740    OUTPUT @Hp4294A;"MEAS COMP"
1750    OUTPUT @Hp4294A;"AUTO"
1760    OUTPUT @Hp4294A;"OUTPDTRC?"
1770    ENTER @Hp4294A USING ";Header$";Header$
1780    ENTER @Binary;Trcc(*)
1790    ENTER @Hp4294A USING ";1 A";Buff$
1800    !
1810    ! Output measurement results to screen and file
1820    !
1830    T_on=TIMEDATE-Start_time
1840    IF Num_res=1 THEN    ! Write headers if first iteration
1850      PRINT "DC CURRENT AND IMPEDANCE DATA"
1880    END IF
1890    FOR I=1 TO Nop_cycles    ! Write a record for each of the sweep points
1900      PRINT USING Img$;T_on,Dc_bias,0,Swp_prm(I),Trca(I,1),Trcb(I,1)
1910      ! Write results to the open file
1920      OUTPUT Wrt$ USING Img2$;T_on,Dc_bias,0,Swp_prm(I),Trca(I,1),Trcb(I,1),Trcc(I,1),Trcc(I,2)
1930    NEXT I
1940    NEXT I
1950    ! Plot LOG(|Z|) versus time, y scale up to 5 E6
1960    ON ERROR GOTO Skip_plot    ! Skip if plotting off scale
1970    IF Num_res=1 THEN
1980      MOVE 590*(T_on/4000),421*(LGT(Trca(2,1))/6.7)
1990    ELSE
2000      MOVE X_old,Y2_old
2010    END IF
2020    DRAW 590*(T_on/4000),421*(LGT(Trca(2,1))/6.7) ! plot |Z| for f=110 Hz
2030 Skip_plot:OFF ERROR
2040    !
2050    Num_res=Num_res+1
2060    X_old=590*(T_on/4000)
2070    Y2_old=421*(LGT(Trca(2,1))/6.7)
2080    RETURN
2090    !
2100 Power_down: !
2110    OUTPUT @Hp4294A;"OUT8 IO 0" ! Turn off TTL O/P to reset relay
2120    OUTPUT @Meter;"OUTP OFF" ! Turn off waveform generator
2130    RETURN
2140    !
2150 End_sequence: !
2160    !
2170    ! Close the results file
2180    ASSIGN @Path_1 TO *
2190    ! WAIT 4
2200    ! backup the results file - caution - Agilent bugs
2210    ! Wrt$="FILC \""&File$&\"".TXT\"",""DISK\"",""&File$&\"".BAK\"",""FLASH\""
2220    ! PRINT Wrt$
2230    ! OUTPUT @Hp4294A;Wrt$
2240    !
2250    ! Power Down the DC O/P at the very end
2260    !
2270    OUTPUT @Hp4294A;"DCO OFF"
2280   OUTPUT @Meter;";OUTP OFF"  ! Turn off waveform generator
2290   OUTPUT @Hp4294A;"OUT8 IO 0"  ! Check TTL O/P is off to reset relay
2300   END
2310   !
2320   ! **********************************************
2330   ! I/O SUBROUTINES ****************************
2340   ! **********************************************
2350   ! File Name Input Function
2360   !
2370 SUB Inp_file_name(Inp_name$)
2380 DIM Inp_char$[30]
2390 ON ERROR GOTO Inp_start
2400 Inp_start: !
2410 PRINT "Input Results File Name (without extension)"
2420 INPUT "File Name? ",Inp_char$
2430 Inp_name$=UPC$(Inp_char$)
2440 IF LEN(Inp_name$)>8 THEN Inp_start
2450 PRINT "Input Name: ",Inp_name$
2460 INPUT "OK? [Y/N]", Inp_char$
2470 IF UPC$(Inp_char$)<>"Y" THEN Inp_start
2480 OFF ERROR
2490 SUBEND
2500 !
Appendix F  iBasic ECIS Script for 4294A

10 ! ******************************************************
20 ! MEASURES IMPEDANCE OF IC ELECTRODES
30 ! LOG X-axis for up to 1 week measurement
40 ! LOG Y-axis
50 ! MEASURES 40 to 1MHz, with 47.9kHz on screen
60 ! No compensation file
70 ! ******************************************************
80 !
90 DIM Mon Osc v(1:11), Mon Osc i(1:11), Mon bias(1:11), Swp Prm(1:11)
100 DIM Trca(1:11,1:2), Trcb(1:11,1:2), Trcc(1:11,1:2)
110 DIM Osc_mode$, Mon bias$, Header$, Buff$, Img$, Img2$, Wrt$
120 DIM Results(1:60,1:7), File$
130 INTEGER Num res, L, Flag
140 REAL Osc_pow, Dc bias, Delay, T on
150 CLEAR SCREEN
160 IF SYSTEM$"SYSTEM ID")="HP4294A" THEN
170 ASSIGN @Hp4294a TO 800
180 ASSIGN @Binary TO 800; FORMAT OFF
190 ELSE
200 ASSIGN @Hp4294a TO 717
210 ASSIGN @Binary TO 717; FORMAT OFF
220 END IF
230 !
240 T on=0
250 Num res=1
260 Osc_mode$="VOLT"
270 Osc_pow=.2
280 Dc_b_rn$="M100"
290 Nop_cycles=11.
300 Img$="DDDDDDD.D,X,D.2DE,X,D.3DE,X,DDDD.DD"
310 Img2$="DDDDDDD.D,X,D.2DE,X,D.3DE,X,DDDD.DD,X,MD.3DE,X,MD.3DE,#"
320 ! Img$ and Img2$ will create a runtime error if expt exceeds 115 days
330 !
340 GCLEAR ! Create a I versus t graph behind the instrument screen
350 ! Draw lines to devide X-axis
360 FOR I=0 TO 10
370 MOVE (590/10)*I,205
380 DRAW (590/10)*I,215
390 NEXT I
400 MOVE 0,210
410 DRAW 590,210
420 !
430 OUTPUT @Hp4294a;";BWFAC 3"
440 OUTPUT @Hp4294a;";FMT LOGY"
450 OUTPUT @Hp4294a;";DISA HIHB"
460 OUTPUT @Hp4294a;";PRES"
470 OUTPUT @Hp4294a;";STAR 40HZ"
480 OUTPUT @Hp4294a;";STOP 1MHZ"
490 OUTPUT @Hp4294a;";POIN ";Nop_cycles
500 OUTPUT @Hp4294a;";POWMOD ";Osc_mode$
510 OUTPUT @Hp4294a;";POWE ";Osc_pow
520 OUTPUT @Hp4294a;";DCMOD VOLT"
530 OUTPUT @Hp4294a;";DCV ";0
OUTPUT @Hp4294a;"DCRNG "$Dc_b_rng$
OUTPUT @Hp4294a;"OMON ON"
OUTPUT @Hp4294a;"BMON CURR"
OUTPUT @Hp4294a;"SWPT LOG"
!
! Request user inputs data file name
CALL Inp_file_name(File$)
! Open file for writing results
CREATE File$&".TXT",5
ASSIGN @Path_1 TO File$&".TXT";FORMAT ON
!

! **************************************
! MAIN PROGRAM START HERE ************
!
Pause until soft key is pressed
OUTPUT @Hp4294a;"USKEY"

Loop0:   !
ON KEY 8 LABEL "START TEST" GOTO Main_test
WAIT .1
GOTO Loop0
!

Main_test:   !
OUTPUT @Hp4294a;"USKEY"
ON KEY 1 LABEL "15s DELAY" GOSUB Delay_15
ON KEY 3 LABEL "1min DELAY" GOSUB Delay_60
ON KEY 4 LABEL "15min DELAY" GOSUB Delay_900
ON KEY 5 LABEL "1hr DELAY" GOSUB Delay_3600
ON KEY 8 LABEL "END EXPT" GOTO End_sequence
Start_time=TIMEDATE
Delay=15 ! readout every 15s

Loop1:   !
GOSUB Measure_dut
SELECT T_on
CASE <900
  Delay=15
CASE <3600
  Delay=60
CASE <43200.
  Delay=3600
CASE >43200.
  Delay=3600
CASE ELSE
  Delay=300
END SELECT
WAIT Delay  ! delay before next readout
GOTO Loop1
!
Delay_15:   !
Delay=15
RETURN

Delay_60:   !
Delay=60
RETURN

Delay_900:   !
Delay=900
RETURN

Delay_3600:   !
Delay=3600
RETURN

GOTO End_sequence
![Image of document page](image-url)
ON ERROR GOTO skip_plot  ! Skip if plotting off scale
IF Num_res=1 THEN
    MOVE 590*(LGT(T_on)/6),421*(LGT(Trca(8,1))/4)
ELSE
    MOVE X_old,Y2_old
END IF
DRAW 590*(LGT(T_on)/6),421*(LGT(Trca(8,1))/4)  ! plot |Z| for f=2.3kHz
Skip_plot:OFF ERROR
!
X_old=590*(LGT(T_on)/6)
Y2_old=421*(LGT(Trca(8,1))/4)
Num_res=Num_res+1
RETURN
!
Power_down: !
OUTPUT @Hp4294a;"OUT8IO 0"  ! Turn off TTL O/P to reset relay
OUTPUT @Wave;"OUTP OFF"  ! Turn off waveform generator
RETURN
!
End_sequence:  !
!
ASSIGN @Path_1 TO *
WAIT 4
backup the results file - caution - Agilent bugs
! Wrt$="FILC "&Inp_name$&".TXT","DISK","&Inp_name$&.BAK","FLASH"
PRINT Wrt$
OUTPUT @Hp4294a;Wrt$
!
Power Down the DC O/P at the very end
!
OUTPUT @Hp4294a;"DCO OFF"
OUTPUT @Hp4294a;"OUT8IO 0"  ! Check TTL O/P is off to reset relay
END
!
*****************************************************************************
I/O SUBROUTINES *************************************************************
*****************************************************************************
SUB Inp_file_name(Inp_name$)
DIM Inp_char$[30]
ON ERROR GOTO Inp_start
!
PRINT "Input Results File Name (without extension)"
INPUT "File Name? ",Inp_char$
Inp_name$=UPC$(Inp_char$)
!
INPUT "OK? [Y/N]",Inp_char$
!
IF UPC$(Inp_char$)<"Y" THEN Inp_start
!
INPUT "OK? [Y/N]",Inp_char$
!
OFF ERROR
Appendix G  Summary of Output


