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An *in vitro* characterisation comparing carbon paste and Pt microelectrodes for real-time detection of brain tissue oxygen

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In vitro characterisation results for O_2 reduction at Pt-based microelectrodes are presented and compared with those for carbon-paste electrodes (CPEs). Cyclic voltammetry indicates a potential of −650 mV *vs*. SCE is required for cathodic reduction at both electrode types, and calibration experiments at this potential revealed a significantly higher sensitivity for Pt (-0.091 ± 0.006 $\mu A mm^{-2} \mu M^{-1}$ *vs.* -0.048 ± 0.002 $\mu A mm^{-2} \mu M^{-1}$ for CPEs). Since Pt electrodes are readily poisoned through contact with biological samples selected surface coated polymers (polyphenylenediamine (PPD), polymethyl methacrylate (PMMA) and Rhoplex®) were examined in biocompatibility studies performed in protein, lipid and brain tissue solutions. While small and comparable decreases in sensitivity were observed for bare Pt, Pt-Rhoplex and PMMA there was minimal change at the Pt-PPD modified electrode for each 24h treatment, including an extended 3 day exposure to brain tissue. The polymers themselves had no effect on the O_2 response characteristics. Further characterisation studies at the Pt-based microelectrodes confirmed interference free signals, no effect of pH and ion changes, and a comparable detection limit (0.08 ± 0.01 μM) and response time (<1 s) to CPEs. Although a significant temperature effect (*ca.* 3% change in signal for each 1 °C) was observed it is predicted that this will not be important for *in vivo* brain tissue O_2 measurements due to brain temperature homeostasis. These results suggest that amperometric Pt electrodes have the potential to be used reliably as an alternative to CPEs to monitor brain tissue O_2 over extended periods in freely-moving animals.

Introduction

Molecular O_2 was one of the first substances detected voltammetrically *in vivo*, both in brain[1,2] and peripheral tissue,[3] as oxygen is an important substrate for many biochemical reactions. Brain tissue O_2 is delivered by the blood and responds to various perturbations including electrical stimulation[4] and neuromediator release.[5] Its turnover rate varys depending on the particular region monitored; the cerebral cortex having a greater rate compared to other areas.[6] While there are currently several methods used for measuring brain O_2 levels (*e.g.* global monitoring using fibre-optic catheters[7–9]) the most common technique remains electrochemical detection using Clark-type electrodes. These directly measure local partial pressure (pO_2) by monitoring O_2 reduction.[2,10,11] A number of mechanisms have been proposed for this reaction including a four-electron transfer which occurs in a single-step without the formation of intermediates:[12]

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

A two-step process has also been reported where hydrogen peroxide is formed as a measurable intermediate:[12]

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$$

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$$

Nobel metals such as Au[13–16] or Pt[2,10,17–19] are predominently the transducer of choice in Clark-type electrodes. While in early reports these tended to be relatively large electrodes (diameters typically in the mm range)[2] they now tend to have dimensions of 3–5 μm.[17] However, while such small dimensions minimise tissue damage associated with implantation, they can result in the concentration of O_2 observed varying due to the orientation of the electrode relative to both blood vessels and metabolically active sites. In addition, metal electrodes have been reported to be highly susceptible to surface poisoning and thus require the use of protecting membranes.[20–22] More recently, carbon-based O_2 electrodes such as carbon fibre (CFE)[23–25] and carbon paste (CPE)[26–28] have been reported. While CFEs have dimensions

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(typically 10 μm) similar to modern Clark-type microelectrodes, CPEs tend to have dimensions greater than the scale of a capillary zone (<100 μm)[29] and as such detect an average tissue O_2 level. However, whilst they do cause greater tissue damage and can be time consuming to construct compared to nobel metal electrodes, they have excellent long-term stability in the *in vivo* environment.[30]

Although Clark-type electrodes are still widely used, and the aplication of carbon electrodes is on the increase, both electrode types clearly have their practical advantages and disadvantages. However, there has to date been no direct comparison of each type in terms of their oxygen reduction characteristics, including sensitivity, selectivity and biocompatability. Thus, in this report we present results of *in vitro* characterisation studies comparing CPEs and Pt electrodes designed for continuous real-time monitoring of brain tissue O_2.

Experimental

Reagents and solutions

The NaCl (SigmaUltra), NaH_2PO_4 (Sigma, A.C.S. reagent) and NaOH (SigmaUltra), KCl (SigmaUltra), $CaCl_2$ (SigmaUltra) and $MgCl_2$ (SigmaUltra) were used as supplied. The monomer *o*-phenylenediamine (*o*PD)[31] was obtained from Sigma, as were bovine serum albumin (BSA) and L-α-phosphatidylethanolamine (PEA). Rhoplex®[20] was obtained from ColourTrend, Celbridge, Ireland. Methyl methacrylate (MMA)[32] was used as supplied from Sigma. Solutions of *o*PD monomer (300 mM) were prepared in PBS. Solutions of BSA and PEA (10%) were prepared in deoxygenated doubly distilled deionised water. A homogenised solution of rat brain tissue was prepared in deoxygenated doubly distilled deionised water. Solutions of Rhoplex® (10%) were also prepared in deoxygenated doubly distilled deionised water.

Compounds used in the interference study were: L-ascorbic acid (AA; A.C.S. reagent, Sigma), dehydroascorbic acid (DHAA; Aldrich), uric acid (UA; sodium salt, Sigma), L-glutathione (oxidised disodium salt, Aldrich) dopamine (DA; hydrochloride, Sigma), 3,4-dihydroxyphenylacetic acid (DOPAC; Sigma), homovanillic acid (HVA; Fluka Biochemika), 5-hydroxytryptamine (5-HT; hydrochloride, Sigma), 5-hydroxyindole-3-acetic acid (5-HIAA; Fluka Biochemika), L-tryptophan (99%, Aldrich), L-cysteine (>98%, Sigma), L-tyrosine (99%, Aldrich). Standard aliquots of 50 μL containing the known extracellular fluid (ECF) concentration of the interferents were prepared using deoxygenated doubly distilled deionised water at the beginning of each experiment to avoid problems with gradual decomposition.

Unless otherwise stated, experiments were carried out in phosphate buffered saline (PBS) solution, pH 7.4 (0.15 M NaCl, 0.04 M NaH_2PO_4 and 0.04 M NaOH). In pH studies, the buffer pH was adjusted to 6.5 and 8.0 using solutions of NaH_2PO_4 and NaOH. Experiments investigating the effects of ion changes (Ca^{2+} and Mg^{2+}) on O_2 sensitivity were performed in artificial cerebrospinal fluid (aCSF): 147 mM NaCl; 4 mM KCl; 1.2 mM $CaCl_2$; and 1 mM $MgCl_2$.[33]

Working electrode preparation

Carbon paste was prepared by thoroughly mixing 0.71 g of graphite powder (1–2 μm, Aldrich) with 250 μL of silicone oil (high temperature, Aldrich).[34] Carbon paste electrodes (8T, 200-μm bare diameter, 250-μm coated diameter) were either supplied by Blue Box Sensors Ltd (Dublin, Ireland) or manufactured in-house from Teflon®-coated silver wire (Advent Research Materials, Suffolk, UK) where the Teflon® insulation was slid along the wire to create an approximately 2-mm deep cavity which was packed with carbon paste using a bare silver wire of same dimensions as a plunger as reported previously.[27] When not in use, all electrodes were stored at 4 °C. Pt-based electrodes were made from (5T, 125-μm bare diameter, 170-μm coated diameter) Teflon®-coated platinum/iridium (Pt/Ir, 90%/10%) wire (Advent Research Materials). The Teflon® insulation was removed from one end of a 6 cm length of wire and this end was soldered into a gold connector for rigidity and electrical connectivity. The active surface of the electrode was created by cutting the opposite end of the wire with a sharp scalpel blade exposing a platinum disk (bare Pt). As previous work has shown that the 90 : 10 Pt-Ir alloy exhibits a range of similar electrochemical properties to those of pure Pt,[35] for simplicity, Pt is used in preference to Pt-Ir throughout the manuscript.

Stock 300 mM solutions of *o*PD monomer were used in the electropolymerisation, carried out amperometrically at +700 mV *vs.* SCE for 30 min to produce Pt-PPD electrodes. Electrodes were rinsed in distilled water post-polymerisation to remove any loosely bound monomer.

Stock 10% solutions of Rhoplex® were used to fabricate Pt-Rhoplex electrodes. The electrode was placed in the 10% solution for an initial 5 min period and then removed and allowed to dry at room temperature for 5 min. Following this drying period, a further 4 layers were deposited on the surface by dip-evaporation producing a total of 5 layers. The electrodes were then left to dry for a minimum of 1 h before being used in experiments.

Undiluted MMA was used to fabricate Pt-PMMA electrodes. The electrode was placed in the pure MMA for a period of 5 s and then removed and allowed to dry at room temperature for a minimum of 1 h before use.

Instrumentation and software

All electrochemical techniques (constant potential amperometry (CPA) and cyclic voltammetry (CV)) were performed using low-noise potentiostats (Biostat II, with a head-stage amplifier from Electrochemical and Medical Systems (EMS), Newbury, UK and Biostat IV, ACM Instruments, Cumbria, UK). Data acquisition was performed with a Dell Dimension L700CXE (Celeron® microprocessor) PC, a PowerLab interface system (ADInstruments Ltd., Oxford, UK) and the software packages LabChart for Windows (Version 6) and EChem for Windows Version 1.5.2 (ADInstruments Ltd.).

All data are presented as mean ± standard error of the mean (SEM), with n = number of electrodes. For analysis of the O_2 calibrations, the O_2 current was plotted *vs.* the O_2 concentration and linear regression performed. The baseline O_2 reduction current was subtracted in all cases and the scale of the O_2 current was inverted to improve the readability of the plotted data. In order to compare data from microelectrodes of different dimensions current values were converted into current density values. The graphical and statistical analysis of data was performed using Microsoft Excel 2007 and the commercial packages

Prism (Version 4.02) and InStat (Graphpad Software Inc., CA, USA). The statistical significance of differences observed was calculated using Student's *t*-tests (two-tailed paired or unpaired observations where appropriate). Values of $P < 0.05$ were considered to indicate statistical significance.

Scanning electron microscopy was performed using a Hitachi S-3200N scanning electron microscope to investigate and compare the different morphological traits of the various microelectrode surfaces.

Experimental protocols

Electropolymerisations, oxygen calibrations and interference studies were performed in a standard three-electrode glass electrochemical cell containing 20 mL PBS at room temperature. A saturated calomel electrode (SCE) was used as the reference electrode and a large Pt wire served as the auxiliary electrode. After application of potential, electrodes were allowed to stabilise for 30 min before measurement began.

For high O_2 concentration calibrations (0–1200 μM), an [O_2] of 0 μM was achieved by deaerating the PBS solution with O_2-free N_2 (BOC Ireland, average O_2 content 2 ppm, maximum O_2 content 5 ppm) for approximately 20 min before commencement of calibration, as it has been observed that sub-micromolar O_2 concentrations can be achieved with constant flushing of the cell with N_2. Secondly, an [O_2] of 240 μM was created by bubbling atmospheric air (from a RENA air pump) through the PBS solution for a similar time period.[36,37] Finally, an [O_2] of 1200 μM was achieved by bubbling pure O_2 gas (BOC Ireland) until the PBS solution became saturated for a similar time period.[36,38] The current was recorded throughout the course of each experiment and steady-states were chosen during the absence of external gaseous agitation.

Low O_2 concentration calibrations (0–125 μM) were performed by adding standard aliquots (+416, +425, +434, +443 and +452 μL) of a saturated O_2 solution (100%), each containing 25 μM O_2, to 20 mL of N_2-saturated PBS. Mixing in this experiment was achieved by placing the electrochemical cell on a magnetic stirrer (IKA MST Mini Magnetic stirrer, Lennox Laboratory Supplies Ltd., Dublin, Ireland) and agitating (*ca.* 10 Hz) the cell solution using a stirring bar (20 mm × 5 mm diameter) for *ca.* 5 s following each injection aliquot.

Calibrations at 37 °C were performed by placing the cell on a temperature regulated magnetic stirrer/hotplate (IKA MST Basic C, Lennox Laboratory Supplies Ltd, Dublin, Ireland). The solution temperature was controlled using a TC1 temperature controller (IKA) which was placed in the solution as near as possible to the working electrode.

Results and discussion

Applied potential for oxygen reduction and oxygen sensitivity

CPEs have been previously used to monitor and measure brain tissue O_2 levels in awake, freely-moving animals. The major advantage that CPEs have in neurochemical studies is their stability over extended periods of continuous recording. This arises from the fact that they are highly resistant to surface poisoning and do not require the use of protecting membranes, as is typical of metal-based electrodes.[30,39,40]

Given the highlighted problems associated with *in vivo* O_2 monitoring using microelectrodes with dimensions in the low μm range (typically ≥ 10 μm) we decided to use Pt electrodes with external diameters of 170 μm (*i.e.* larger than the scale of a capillary zone). This is smaller than the diameter of CPEs (typically 250 μm) and close to the threshold diameter of 150 μm (reported by O'Neill and co-workers[41]) above which gliosis becomes detectable as evidenced by the release of uric acid.

We initially compared the O_2 reduction characteristics of both electrode types using cyclic voltammetry at a scan rate of 100 mV/s. For CPEs a clear O_2 reduction peak was observed at *ca.* −600 mV with the foot of the wave occurring at −300 mV. At Pt, the foot of the wave occurs at −200 mV with no defined cathodic peak nor a limiting current in the potential range available. Thus, in order to compare differences in charge transfer kinetics we calculated the O_2 reduction slope as di/dV by subtracting successive current readings and dividing by the voltage difference; the potential of maximum slope,[42] $E_{s,max}$, was assigned to the lower potential point of the pair which showed the highest value along the wave. From voltammograms, $E_{s,max}$ was calculated as −0.50 V for CPEs and −0.24 V for bare Pt indicating that the rate constant for charge transfer is greater at Pt compared with CPEs (Fig. 1). Reduction of O_2 at carbon is known to be a two-electron process producing H_2O_2 (see Introduction). Since the direct reduction[43] (and oxidation[24,44]) of H_2O_2 is severely inhibited at carbon electrode surfaces the rate-limiting step is the initial one-electron transfer followed by protonation of the superoxide ion and further reduction.[44] On Pt (in neutral/alkaline solutions) while the H_2O_2 intermediate may be reduced further, the major reaction path is complete (one-step) four-electron transfer without peroxide formation.[45] Thus, in CPA

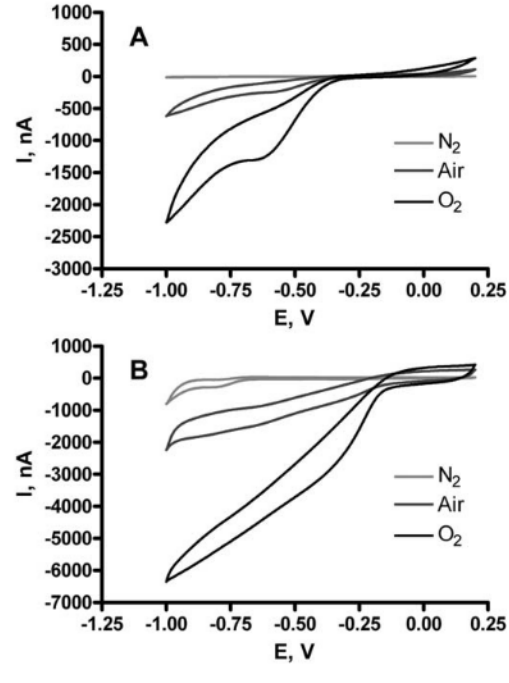

Fig. 1 Typical cyclic voltammograms in the range +0.2 to −1.0 V *vs.* SCE recorded at 100 mV s^{-1} with (A) CPEs and (B) Pt microelectrodes in the presence of N_2, air and O_2.

experiments we decided to use a potential of −650 mV for all O_2 calibrations at both electrode types.

In order to determine the O_2 sensitivity of CPEs and Pt electrodes operating at −650 mV, calibrations were performed over a conventionally reported 0–1200 μM O_2 range (N_2, air and O_2-saturated PBS).[28,38,40] It takes approximately 10 min for the PBS buffer solution to reach a new level of saturation for each gaseous concentration (Fig. 2). The decreases in the observed signal during the quiescent periods in the calibrations are due to the removal of the bubbling artefact associated with forced convection from the introduction of the different gases. Oxygen calibrations with CPEs displayed good linearity ($R^2 - 0.999$) and sensitivity (-1.51 ± 0.05 nA μM^{-1}, $n - 8$) while the response was linear ($R^2 - 0.995$, $n - 18$) with a slope of -1.12 ± 0.08 nA μM^{-1}, $n - 18$ for Pt electrodes (Fig. 2, *insets*).

As the distribution of concentrations reported for brain tissue ranges from 40 μM to 80 μM, calibrations were also performed at low O_2 concentrations (0–125 μM O_2, Fig. 3). Oxygen calibrations with CPEs produced a similar, linear sensitivity (-1.09 ± 0.03 nA μM^{-1}, $R^2 - 0.998$, $n - 4$), while for Pt electrodes the response was also linear ($R^2 - 0.979$) with a similar sensitivity (-1.43 ± 0.05 nA μM^{-1}, $n - 4$) and subsecond response times for both electrode types (Fig. 3, *insets*).

Due to the different physical dimensions of the two electrode types, current density values were calculated in order to compare response characteristics. The sensitivity of the Pt electrode was approximately double that of the CPE: -0.091 ± 0.006 μA mm^{-2} μM^{-1}, $n - 18$, $R^2 - 0.995$ and -0.048 ± 0.002 μA mm^{-2} μM^{-1}, $n - 8$, $R^2 - 0.999$ respectively.

The limit of detection was similar for both electrode types: bare Pt, 0.08 ± 0.01 μM ($n - 14$); CPEs, 0.08 ± 0.01 μM ($n - 16$). The latter is in agreement with previously published data for CPEs.[40]

Although it is easier to construct a bare Pt electrode compared to a CPE it is widely recognised that metal electrodes need to be coated with a protective membrane primarily to overcome poisoning issues associated with implantation in biological tissue.[21,22] While traditional Clark-type electrodes utilised physical membrances (*e.g.* cellophane) more recently developed microelectrodes are coated with polymers applied through various methods including droplet evaporation, dipping and electropolymerisation. We thus examined the effects of three such polymers (PPD, Rhoplex® and PMMA) applied to the bare Pt electrode surface.

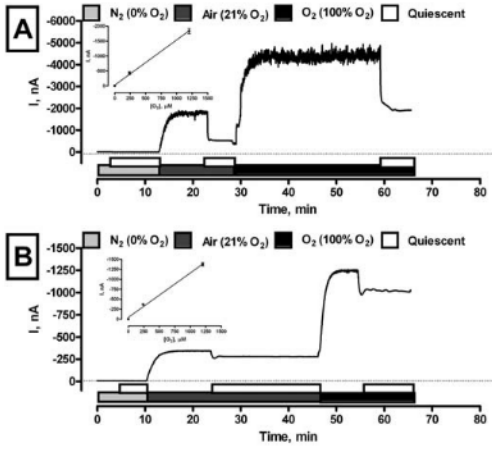

Fig. 2 Typical current-time profiles for O_2 calibrations (0 1200 μM; N_2, air and O_2 saturation) at (A) CPEs and (B) bare Pt electrodes performed using constant potential amperometry (CPA) at −650 mV (*vs.* SCE) in PBS, pH 7.4. *Insets*: Current-concentration profile for quiescent steady-state (A) CPE (n – 8) and (B) bare Pt O_2 currents (n – 18).

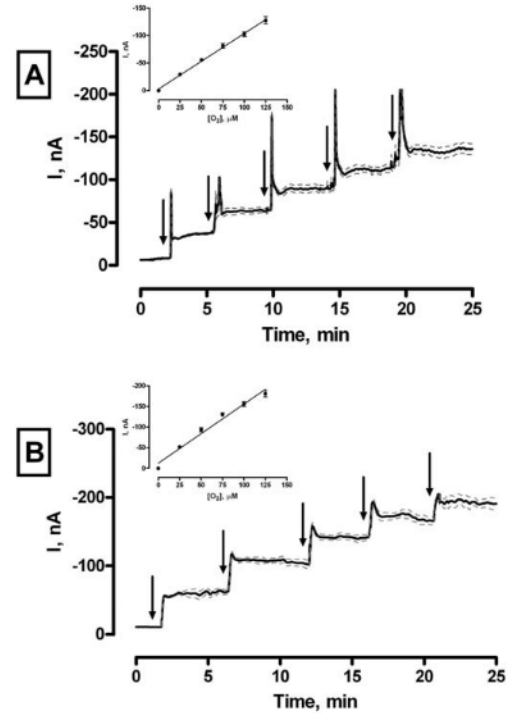

Fig. 3 Current-time profiles for O_2 calibrations (0 125 μM) at (A) CPEs and (B) bare Pt electrodes performed using constant potential amperometry (CPA) at −650 mV (*vs.* SCE) in PBS, pH 7.4. Arrows indicate injections (+416, +425, +434, +443 and +452 μL) of a saturated O_2 solution (1.2 mM) yielding concentrations of 25, 50, 75, 100 and 125 μM O_2. Hashed grey lines represent the SEM. *Insets*: Current-concentration profile for the quiescent steady-state (A) CPE (n – 4) and (B) bare Pt O_2 currents (n – 4).

Initially, the structural appearance of the modified electrodes was examined using electron microscopy. Fig. 4 shows examples of bare Pt, Pt-PPD, Pt-Rhoplex and Pt-PMMA surfaces at magnifications of x500/600 and x12000. Bare Pt shows a fine striated pattern which appears parallel to the direction of the cut. The Pt-PPD surface shows a grainy morphological structure that appears to adhere to the individual striations on the metal suface and is consistent with formation of a thin (*ca.* 5–10 nm) insulating PPD layer.[46] The Pt-Rhoplex surface appears quite different to the bare metal with a thick, smooth polymer layer covering the metal surface and aggregating at the metal-Teflon® interface. The Pt-PMMA surface appears quite similar to the Pt-PPD surface with a grainy morphology that clearly covers the bare metal without obscuring the striated metal surface.

Significant changes in sensitivity compared to the bare metal electrode were not observed upon calibration of the modified electrodes over the conventional 0–1200 μM O_2 range: Pt-PPD, -0.95 ± 0.09 nA μM^{-1}, $n - 17$, $R^2 - 0.991$ ($P - 0.1663$); Pt-

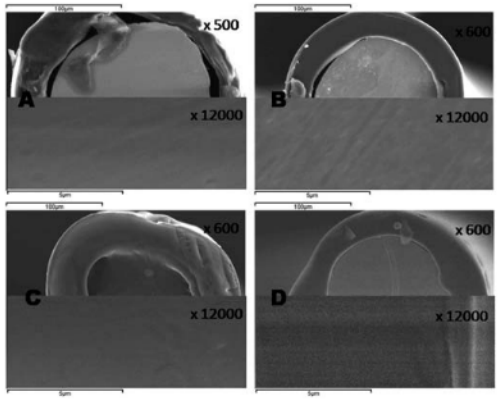

Fig. 4 Scanning electron micrographs at low (top) and high (bottom) magnification of the surface of (A) bare Pt, (B) Pt-PPD, (C) Pt-Rhoplex and (D) Pt-PMMA modified electrodes.

Rhoplex, -1.02 ± 0.10 nA μM^{-1}, $n - 18$, $R^2 - 0.991$ ($P - 0.4403$); Pt-PMMA, -1.06 ± 0.06 nA μM^{-1}, $n - 12$, $R^2 - 0.993$ ($P - 0.5893$) indicating that the presence of these polymer coatings does not affect the diffusion of oxygen to the active surface of the electrode. Similarily, sensitivities obtained from calibrations performed with the modified electrodes at physiologically relevant concentrations (0–125 μM O_2) did not differ significantly from the bare electrode response: Pt-PPD, -1.34 ± 0.13 nA μM^{-1}, $n - 10$, $R^2 - 0.9812$ ($P - 0.6797$); Pt-Rhoplex, -1.37 ± 0.06 nA μM^{-1}, $n - 4$, $R^2 - 0.9896$ ($P - 0.4715$); Pt-PMMA, -1.35 ± 0.06 nA μM^{-1}, $n - 4$, $R^2 - 0.9893$ ($P - 0.3452$).

Biocompatibility

CPEs are known to be modified after contact with brain tissue[42,47] producing stable biocompatible electrodes. Since it is generally accepted that Pt electrodes are readily poisoned through contact with biological samples we decided to test the effect of brain tissue and its component parts on the sensitivity of the Pt-based electrodes. The bare Pt and the modified electrodes were exposed to solutions of proteins (BSA), lipids (PEA) and brain tissue for 24 h in order to test the biocompatibility of the electrodes. The results are summarised in Table 1.

While there was no effect on the responses found for bare Pt and Pt-PPD small but non-significant decreases were observed for Pt-Rhoplex and Pt-PMMA after exposure to BSA and brain tissue. Further exposure to brain tissue (homogenised solution) for 3 days resulted in a drop in sensitivity for bare Pt (values comparable to Pt-Rhoplex and Pt-PMMA) with Pt-PPD being the most stable (compared to the untreated electrodes): bare Pt (-0.90 ± 0.03 nA μM^{-1}, $R^2 - 0.9987$, $n - 4$, $P - 0.2192$); Pt-PPD (-0.89 ± 0.10 nA μM^{-1}, $R^2 - 0.9869$, $n - 3$, $P - 0.7901$); Pt-Rhoplex (-0.85 ± 0.10 nA μM^{-1}, $R^2 - 0.9874$, $n - 4$, $P - 0.4496$); Pt-PMMA (-0.87 ± 0.07 nA μM^{-1}, $R^2 - 0.9859$, $n - 4$, $P - 0.1141$). There were no changes in sensitivity of the CPEs (-1.51 ± 0.05 nA μM^{-1}, $n - 8$) when exposed to brain tissue for 1 and 3 days: -1.55 ± 0.18 nA μM^{-1}, $n - 21$, $P - 0.9069$ and -1.51 ± 0.14 nA μM^{-1}, $n - 26$, $P - 0.9938$ respectively. Similar biocompatibility characteristics have been reported previously for CPEs.[40] Similar stability observations were previously found for Pt-PPD based glucose biosensors.[46]

Selectivity

The selectivity of Pt-based electrodes for O_2 relative to a selected range of potential interferents[40] present in brain extracellular fluid (ECF) was characterised *in vitro*. The compounds tested were L-ascorbic acid (AA) and the neurotransmitters dopamine (DA) and 5-hydroxytryptamine (5-HT), their metabolites 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), and other electroactive species such as L-tyrosine, L-cysteine, L-tryptophan, L-glutathione, dehydroascorbic acid and the purine metabolite uric acid (UA). Calibrations were performed by adding standard aliquots (50 μL) containing ECF concentrations of each of the interferents, with a typical example shown in Fig. 5. Small current increases (<1.5 nA) were observed immediately after addition on each of the electrode types, with the signal returning to baseline within the quiescent period preceeding the next injection. Current values for ascorbic acid (500 μM), an endogenous electroactive species present at high concentrations relative to other interferents,[48] provide a representative example of

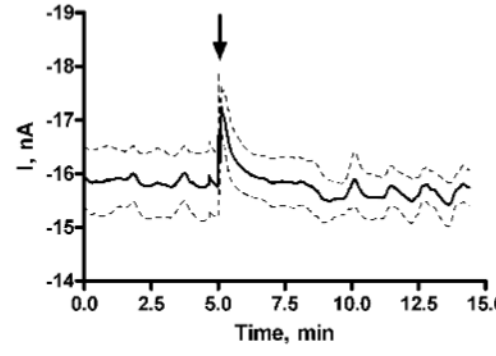

Fig. 5 A typical *in vitro* current-time response for CPA (−650 mV *vs.* SCE) at Pt-PPD electrodes ($n - 4$) in N_2 saturated PBS (pH 7.4) for addition of interferent species. Arrow indicates the injection of ascorbic acid (500 μM). Hashed (grey) lines represent the SEM. Typical background current (Pt-PPD, -15.91 ± 0.52 nA, $n - 4$).

Table 1 Sensitivity values for bare Pt, Pt-PPD, Pt-Rhoplex and Pt-PMMA electrodes, untreated and treated with BSA, PEA and brain tissue for 24 h. Number of electrodes in parentheses

	Bare Pt (nA μM^{-1})	Pt-PPD (nA μM^{-1})	Pt-Rhoplex (nA μM^{-1})	Pt-PMMA (nA μM^{-1})
Untreated	-1.12 ± 0.08 (18)	-0.95 ± 0.09 (17)	-1.02 ± 0.10 (18)	-1.06 ± 0.06 (12)
BSA	-1.06 ± 0.13 (4) $P > 0.74$	-0.91 ± 0.10 (4) $P > 0.83$	-0.91 ± 0.10 (4) $P > 0.62$	-0.91 ± 0.08 (6) $P > 0.16$
PEA	-1.00 ± 0.09 (4) $P > 0.50$	-0.89 ± 0.08 (4) $P > 0.75$	-0.98 ± 0.13 (3) $P > 0.87$	-0.97 ± 0.04 (4) $P > 0.42$
Tissue	-1.00 ± 0.10 (3) $P > 0.56$	-0.91 ± 0.13 (3) $P > 0.86$	-0.90 ± 0.12 (3) $P > 0.64$	-0.89 ± 0.04 (4) $P > 0.13$

the typical signals recorded at the various modified electrodes: bare Pt (-0.08 ± 0.04 nA, $n-4$); Pt-PPD (-1.32 ± 0.81 nA, $n-4$); Pt-Rhoplex (-1.56 ± 0.36 nA, $n-4$); Pt-PMMA (-0.11 ± 0.50 nA, $n-4$). In direct comparison with the actual O_2 sensitivity values for ECF concentrations, these slight drifts in current are negligible and are merely a convective artefact associated with the addition of the respective aliquot. A slight baseline drift was observed over the course of all experiments and is attributable to maintaining the N_2 atmosphere over the electrolyte solution. Similar selectivity characteristics have been reported previously for CPEs.[40] Consequently, these electrodes will have signals in the *in vivo* environment that are not influenced by the milieu of interferents present.

Effect of ion changes, temperature and pH

The media-dependence of redox reactions for several physiologically important electroactive species have previously been studied by several groups.[49–51] For example, fast cyclic voltammetry (FCV) sensitivities to DA and 5-HT at CFEs have been reported to be 2–3-fold higher in non-physiological phosphate or HEPES-buffered saline compared to artificial cerebrospinal fluid (aCSF) which more accurately reflects the ionic composition of the brain.[49,50] This is important when one considers that *in vivo* extracellular ion concentrations are in a continuous state of flux and can change significantly with acute or chronic pertubations to normal physiology. For example, $[Ca^{2+}]$ can fall under conditions of both electrical stimulation (*e.g. ca.* 80 μM for 10 Hz at 10 s)[49] and intense tissue depolarisation (*e.g.* >1 mM for anoxic depolarisation or spreading depression).[51] In addition, changes in pH may occur during physiological experiments *in vivo*[24] (these could also affect the cathodic reduction of O_2 which involves proton transfer), and classical membrane covered noble metal-based O_2 electrodes tend to have significant temperature dependence (the signal increasing by 1–6 °C for a rise of 1 °C[45,52]). We thus decided to test the effect of ion, pH and temperature changes on the bare Pt O_2 response.

O_2 sensitivities for calibrations performed in the range 0–1200 μM O_2 in the presence and absence of Ca^{2+} and Mg^{2+} were similar: aCSF (-0.98 ± 0.04 nA μM^{-1}, $R^2 - 0.9947$ $n-8$); aCSF no Ca^{2+} (-0.99 ± 0.03 nA μM^{-1}, $R^2 - 0.9917$, $n-8$, $P-0.8365$); aCSF no Mg^{2+} (-1.02 ± 0.06 nA μM^{-1}, $R^2 - 0.9953$, $n-8$, $P-0.4926$) indicating that ion changes in physiological media will not affect the bare Pt O_2 reduction signal. Furthermore, the sensitivity of the bare Pt electrodes did not differ significantly between PBS (-1.12 ± 0.08 nA μM^{-1}, $R^2 - 0.995$, $n-18$) and aCSF (-0.98 ± 0.04 nA μM^{-1}, $R^2 - 0.995$, $n-8$, $P-0.2734$).

It is well recognised that the mechanism of O_2 reduction is influenced by the electrode material as well as the solution pH used in the experimental system.[53] The electrochemical reduction of oxygen on noble metal-based electrodes has received a long-standing interest and has been extensively studied. As already outlined, O_2 reduction to water at Pt has been proposed to take place either *via* a "direct" four-electron transfer or a "series" mechanism involving a two-electron reduction to H_2O_2, with formation of H_2O_2(ads), followed by its reduction to water. Wang *et al.* showed the two mechanisms operating in parallel at Pt(111), but with evidence that the direct four-electron pathway is the dominant one.[54] In contrast, the reduction of O_2 at carbon electrodes proceeds *via* the two-electron process as reduction of H_2O_2 is severely inhibited at carbon surfaces.[40] As both mechanisms involve proton transfer the reaction can be affected by pH. Previous reports for carbon-based electrodes found that for pH 12–14 the reduction of O_2 appears to be independent of pH, but as pH decreases the reduction becomes pH dependent.[44,55] For Pt-based electrodes it has been reported that the oxygen reduction peak potential shifts by −50 mV per pH unit for cyclic voltammetry carried in air-saturated phosphate buffers of pH 4, 7, and 11.[56] To test the pH sensitivity of our bare Pt microelectrodes, the buffer solution pH was changed from the standard physiological 7.4 to 6.5 and 8.0. No significant difference was observed in the O_2 sensitivity at the various pH values: pH 7.4 (-1.12 ± 0.08 nA μM^{-1}, $R^2 - 0.995$, $n-18$); pH 6.5 (-1.06 ± 0.04 nA μM^{-1}, $R^2 - 0.9987$, $n-8$, $P-0.6117$); pH 8.0 (-1.07 ± 0.04 nA μM^{-1}, $R^2 - 0.9951$, $n-8$, $P-0.6946$). Similar findings have recently been reported for O_2 reduction at CPEs.[40]

The temperature dependence of classical O_2 electrodes has been primarily attributed to the variation in solubility of the gas in the membrane with temperature and it is generally recommended that the operation of any O_2 electrode should be carried out under thermostatic conditions. This can be achieved by either controlling the sample solution temperature or by regulating the temperature of the electrode itself so that the membrane and diffusion layers are at a constant temperature throughout an experiment.[57–59] If thermostatic conditions are not feasible, temperature effects must be compensated for in order to obtain accurate O_2 measurements. One example of this type of compensation is to measure the temperature of the solution near the electrode with a thermocouple and use a calibration curve to determine the dissolved oxygen concentration from the current response. As our *in vitro* experiments are routinely performed at room temperature (*ca.* 22.5 ± 0.2 °C), we therefore examined the effect of increasing temperature on the Pt O_2 sensitivity. A significant difference was observed in the sensitivity of the electrodes calibrated at room temperature (-1.12 ± 0.08 nA μM^{-1}, $R^2 - 0.995$, $n-18$) and those calibrated at the physiologically relevant 37 °C (-1.57 ± 0.06 nA μM^{-1}, $R^2 - 0.9963$, $n-12$, $P-0.0002$). This represents an approximate 3% change in signal for each 1 °C and is in contrast to CPEs where no temperature effect is observed.[40] Although brain temperature and brain temperature homeostasis are poorly studied issues it is widely accepted that brain tissue is exceptionally sensitive to heat, with pathological changes occurring when there is a 3–4 °C increase above normal baseline (37 °C). As such temperature is tightly regulated by the circulatory system and normal fluctuations associated with activity (*e.g.* physical exercise) are usually within ± 1 °C of baseline.[60] Given that a 3% alteration in signal represents a change in current of *ca.* 0.03 nA we would predict that temperature compensation is not required for *in vivo* measurements using Pt O_2 electrodes in the brain. Indeed, compensation mechanisms such as that described above would not be practical with such implanted electrodes.

Conclusions

Pt-based electrodes were fabricated that reliably monitor O_2 at a constant potential of −650 mV *vs.* SCE. *In vitro*

characterisation studies indicate interference free signals, no effect of pH and ion changes, and a comparable detection limit and response time to CPEs, but a higher sensitivity. While a significant temperature effect was observed it is predicted that this will not be important for brain tissue O_2 measurements and that temperature compensation will not be required. Interestingly, despite a literature consensus indicating Pt electrode poisoning in biological tissues, results from biocompatibility studies comparing bare Pt *vs.* membrane-coated Pt electrodes point to minimal evidence of fouling of the active surface. As we have recently reported *in vivo* characterisation results for CPEs[40] future work will concentrate on the *in vivo* characterisation of the Pt-based electrodes in freely-moving animals, in order to determine if these positive *in vitro* characteristics of good sensitivity, selectivity and stability are transferable to the target *in vivo* environment.

The significance of these results is that Pt-based electrodes can potentially be used reliably as an alternative to CPEs for continuous monitoring of brain tissue O_2, allowing for easier manufacture of smaller electrodes which cause less tissue damage, and can be targeted at brain regions where the dimensions of CPEs may limit their application.

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