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In vitro development and *in vivo* application of a platinum-based electrochemical device for continuous measurements of peripheral tissue oxygen

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ABSTRACT

Acute limb ischaemia is caused by compromised tissue perfusion and requires immediate attention to reduce the occurrence of secondary complications that could lead to amputation or death. To address this, we have developed a novel platinum (Pt)-based electrochemical oxygen (O_2) device for future applications in clinical monitoring of peripheral tissue ischaemia. The effect of integrating a Pt pseudo-reference electrode into the O_2 device was investigated *in vitro* with an optimum reduction potential of −0.80 V. A non-significant ($p = 0.11$) decrease in sensitivity was recorded when compared against an established Pt-based O_2 sensor operating at −0.65 V. Furthermore, a biocompatible clinical sensor (ClinOX) was designed, demonstrating excellent linearity ($R^2 = 0.99$) and sensitivity (1.41 ± 0.02 nA μM^{-1}) for O_2 detection. Significant rapid decreases in the O_2 current during *in vivo* ischaemic insults in rodent limbs were reported for Pt-Pt ($p < 0.001$) and ClinOX ($p < 0.01$) and for ClinOX ($p < 0.001$) in porcine limbs. *Ex vivo* sensocompatibility investigations identified no significant difference ($p = 0.08$) in sensitivity values over 14 days of exposure to tissue homogenate. The Pt-Pt based O_2 design demonstrated high sensitivity for tissue ischaemia detection and thus warrants future clinical investigation.

1. Introduction

Oxygen (O_2) is of critical importance in a myriad of physiological processes. Thus, timely and accurate monitoring techniques are essential to assist clinicians with potential lifesaving decisions. Historically, oxygenation has been monitored subjectively by clinical assessment due to the paucity of techniques to accurately evaluate precise tissue oxygen levels. Over the past 60 years, measuring and monitoring methods have been developed to quantify oxygen supply and, more recently, to continuously assess the oxygenation of blood and tissues both invasively and noninvasively [1,2]. Currently, the traditional gold standard methods of O_2 measurement performed in hospitals are pulse oximetry (PulsOx) and arterial blood gas analysis (ABG). PulsOx was developed in 1972 by Japanese engineers [3] who noted the pulsatile components of the absorbance of red and infrared light transmitted through tissue were related to arterial haemoglobin saturation [4]. However, this method is an indirect marker of tissue perfusion and functionality is lost in the absence of a pulse wave [5]. ABG machines revolutionised critical patient care following their introduction in the late 1950's [2] as blood samples could be analysed for a range of parameters including blood gases, haemocrit, electrolytes, metabolites, and co-oximetry. However, significant drawbacks include the time lag associated with single point measurements and the deterioration of blood samples, culminating in false readings and misdiagnoses. Moreover, both methods are unsuitable for continuous real-time O_2 measurements in peripheral tissue due to their reliance on vascular factors.

No gold standard currently exists for the early diagnosis of trauma- or thrombosis-induced complications. Acute limb ischaemia (ALI), a common cause of morbidity and mortality, is defined as a restriction in blood flow to the extremities that causes a shortage of the O_2 and glucose required for cellular metabolism. ALI pathophysiologies require timely surgical intervention to restore blood perfusion to the impacted tissue. For example, acute compartment syndrome (ACS) is a very serious condition that results from increased pressure within a muscle compartment following trauma, which can lead to muscle and nerve damage and compromised blood flow unless detected early. When the intramuscular tissue pressure becomes higher than the blood pressure within capillaries, the capillaries collapse and disrupt blood flow, O_2, and nutrient delivery to nerve and muscle cells that renders them ischaemic [6–9]. Compartment syndrome is a time-critical medical emergency, in which irreversible nerve and muscle damage can occur after just six hours of increased intra-compartmental pressure. Recovery from tissue ischaemia complications is largely dependent on tissue

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microcirculation [10] in the smallest blood vessels, including arterioles, capillaries, and venules, which are responsible for tissue oxygenation and, ultimately, tissue (and organ) health [11].

Blood perfusion is commonly assessed by non-invasive Laser Doppler Imaging (LDI), which measures blood flow through backscattering a laser beam that interacts with moving red blood cells [12,13]. While the non-invasive nature of LDI is highly desirable, it suffers from a lack of standardisation, poor temporal resolution, and can only penetrate tissue to a maximum depth of 10 mm [10]. More recently, near infrared spectroscopy (NIRS) has demonstrated excellent clinical utility that has facilitated clinical diagnoses [14–16]. NIRS utilises light waves ranging from 680 to 800 nm to measure tissue O_2 saturation by light absorption of oxygenated and deoxygenated haemoglobin [17] with increased tissue penetration up to a depth of 15 mm [5]. However, this indirect measure of tissue oxygenation with varied predictability for any clear effect magnitude or ischemic threshold has limited the clinical applicability of NIRS [18]. Furthermore, the probe has depth measurement restrictions in that superficial muscle readily absorbs the light, while deep muscles are difficult to isolate [18].

Molecular O_2 electrochemical detection was first reported by Clark approximately 60 years ago with a gold or platinum (Pt) working electrode and an Ag/AgCl reference electrode in a KCL electrolyte encased within an O_2 permeable membrane [19,20]. Over the years, Clark-type electrodes have become the gold standard for measuring levels of O_2 in tissue [21]. In fact, the aforementioned ABG machine utilises a Clark O_2 electrode. In addition the Licox® O_2 probe, which is also based on the Clark design, is the only invasive O_2 system currently approved for clinical use, particularly for cerebral O_2 monitoring [22]. The continuous real-time monitoring with the Licox® O_2 probe in traumatic brain injury patients has gained significant traction in the last decade [21,23]. Moreover, a number of recent studies have reported applications within skeletal muscle [24–26] and the feasibility of continuous tissue O_2 measurements has been demonstrated in humans following tibia fracture [27]. Hansen et al. confirmed that the level of muscle O_2 responds rapidly to ischemia and correlates with biochemical markers of muscle ischemia, including ATP and pH [25]. The Licox® O_2 probe was designed with brain monitoring as its core application and the development of a more suitable intramuscular sensor to directly measure O_2 levels would represent a key advancement in diagnosing tissue ischemia complications, such as compartment syndrome. The onset of post-trauma compartment syndrome symptoms can range from 2 h to 6 days, and delayed diagnoses could result in permanent nerve damage, contractures, or amputation, requiring extensive follow-up consultations and procedures for otherwise healthy patients. Due to the severe health implications of acute ischaemia, the development of a medical device to detect early onset in "at risk" patients by measuring muscle oxygenation following a high impact trauma would be advantageous and extremely beneficial for clinicians.

Different electrode substrates have been utilised for the electrochemical detection of O_2, including noble metals such as Pt [21,28] and Au [29,30] and carbon-based materials comprising glassy carbon [30], carbon fibre (CFE) [31,32], and carbon paste (CPE) [28,33]. CFEs are advantageous as tissue damage is minimal following *in vivo* implantation; however, variations in the detection of O_2 concentrations can result from electrode placement relative to blood vessels and metabolically active sites [28]. Furthermore, their fragility renders them unsuitable for clinical monitoring. On the other hand, CPEs demonstrate excellent long-term stability once implanted [33], however, there are biocompatibility concerns related to the gradual leeching of oil and paste upon contact with lipids in tissue [34]. Pt is the preferred choice of electrode substrate in the majority of electrochemical O_2 sensors due to its excellent ability to electrocatalyse the reduction of molecular O_2 through a single step, four-electron process to H_2O [21,28]. Furthermore, its biocompatibility makes it an ideal candidate for acute and chronic implantation in the human body [35]. It is a major component of many medical devices in use worldwide for assessing conditions such as heart disease, stroke, neurological disorders, and chronic pain [35]. This work describes the *in vitro* development of a novel and implantable Pt-based electrochemical sensor (ClinOX) for monitoring peripheral tissue O_2 using a sensing component suitable for future patient monitoring. The *in vitro* performance of this ClinOX design was investigated and compared against a pre-clinical Pt design. The efficacy of the different designs at measuring peripheral tissue ischaemia was investigated in small and large animal models. Finally, the effect of long-term exposure to muscle tissue homogenate on Pt sensitivity was investigated *ex vivo*. The work described demonstrates the feasibility of a Pt-based electrochemical device for the continuous real-time monitoring of peripheral tissue O_2 in humans in the future.

2. Materials and methods

2.1. Chemicals and solutions

All reagents used in phosphate buffered saline (PBS), *i.e.* sodium chloride (NaCl), sodium hydroxide (NaOH) and sodium hydrogen phosphate (NaH_2PO_4), and clinical interference studies, *i.e.* acetaminophen and acetylsalicyclic acid, were purchased from Sigma Aldrich Chemical Co. (Dublin, Ireland).

2.2. Electrode manufacture

The electrodes used in this study were manufactures as follows:

2.2.1. Platinum (Pt) disk working electrodes (O_2 electrode)

Pt disk electrodes were made from Teflon®-insulated Platinum/Iridium (Pt/Ir 90%/10%) wire (127 μm bare diameter, 203 μm coated diameter (5 T), Science Products GmbH, Hofheim, Germany). The electrodes were 5 cm in length and were prepared by carefully cutting 2 mm of the Teflon® insulation from one end of the wire. A gold electrical contact (*in vitro*: Fine Science Tools GmbH, Heidelberg, Germany; *in vivo*: Bilaney Consultants, Sevenoaks, UK) was soldered for rigidity to the bare end to enable connection with electrochemical instrumentation. A fresh surface was cut at the opposite end of the wire to generate an active disk surface for electrochemical reactions.

2.2.2. Pt cylinder electrodes

Pt cylinder electrodes were manufactured in a similar manner, except that a cylinder surface was prepared by removing 5 mm of the Teflon® insulation from the active end of the wire. These cylinder electrodes served as pseudo-reference electrodes (PRE) and auxiliary electrodes for Pt-Pt O_2 sensors.

2.2.3. Pt-SCE O_2 sensor

This sensor design consisted of a Pt disk O_2 electrode, a saturated calomel electrode (SCE) as the reference electrode, and a Pt rod that acted as the auxiliary electrode.

2.2.4. Pt-Pt O_2 sensor

The sensor design comprised a Pt disk O_2 electrode, a Pt cylinder PRE, and a Pt cylinder auxiliary electrode.

2.2.5. Clinical O_2 sensor (ClinOX)

The sensor was manufactured in a two-step process as represented schematically in Figure SM2. A nylon tip was constructed using injection moulding (Clada Medical Devices, Galway, Ireland). A channel was incorporated into this nylon tip to facilitate a 1–2 mm length of 500 μm bare Pt rod (Advent Research Materials, Oxford, UK). A 25 cm length of Teflon® insulated 100 μm copper wire (Goodfellow, Huntingdon, UK) was soldered to the Pt rod following removal of 2 mm Teflon® insulation at one end. This construct was then fed through the channel and the Pt rod was secured using epoxy with 0.1–0.2 mm protruding from the nylon tip and the copper wire from the opposite end. A scalpel