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Electrochemical monitoring of subcutaneous tissue pO_2 fluctuations during exercise using a semi-implantable needle electrode

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Abstract

Semi-implantable needle oxygen electrodes were used for forearm subcutaneous monitoring in human subjects undertaking high intensity cycling and fist clenching exercise. pO_2 variations in the range between 40 and 100 mmHg oxygen were seen. Superimposed on these were paradoxical rises in subcutaneous pO_2 , of up to 100 mmHg which paralleled the scale of the exercise. This was indicative of increased blood flow through skin. Triton X-100 incorporated into the sensor polyurethane membranes helped to give faster responses and reduced the possibility of biofouling and drift. The sterilizable system, free from internal electrolyte film appears promising for future clinical monitoring.

Keywords: pO_2 sensor, Needle electrode, Tissue oxygenation, *in vivo* monitoring, Exercise, Oxygen fluctuation.

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1. Introduction

Tissue oxygenation state is the ultimate enabler of healthy cellular metabolism, and in mammalian systems anaerobic metabolism is only possible during short periods as a response to extreme physiological stress caused by limited oxygen supply [1]. This is typically when there is central or peripheral circulatory shut down, or during high intensity exercise when any shunting of aerobic metabolic pathways is a feedback response to a lack of oxygen [1]. In clinical situations radical oxygenation failure is seen classically in cardiovascular shock, rapid blood loss, injury, critical illness or sepsis [2]. There may also be failures of local circulation compromising specific peripheral tissues as in peripheral vascular disease and late diabetes [3]. Measurement of tissue oxygen partial pressure (pO_2) has the potential to provide a metabolic 'short-hand' of the balance between the adequacy of a blood supply and cellular oxygenation demand [2].

Arterial blood gases [4,5] are the mainstay of clinical evaluation, but may not be able identify local changes or where wider peripheral small vessel or other disseminated changes take place. Tissue pO_2 , however, is non-accessible in routine clinical practice, and more the target of fundamental physiological studies. Experimental work, for example, has shown that subcutaneous tissue pO_2 in lean and obese patients is similar but diverges in surgical patients, reflecting co-morbidities and other acute care clinical management outcomes [6,7]. Contrasting outcomes in arterial blood, as against peripheral tissue oxygen, was evident in the post-injury hypovolaemic shock state where though blood pO_2 indicated recovery, whilst tissue pO_2 revealed persistent hypoxia pointing to the need to maintain longer term care and optimize support procedures to secure full recovery [8]. An animal study [9] showed that during the development of haemorrhagic shock, there was early divergence of blood and subcutaneous tissue pO_2 . The electrode used in this study

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is a hydrophobic polyurethane barrier membrane reprising the Clark oxygen electrode model. pO_2 change was readily followed, but a substantial delay in tracking of oxygen change *in vitro*, of the order of 15 mins may have dampened some of the minor variations present *in vivo*. Electrodes implanted in sacral tissue in a human study as part of an investigation of pressure ulcers was able to track pO_2 during and after applied mechanical pressure [10].

The general area of tissue oxygen measurement has been reviewed by Carreau *et al* [11]. Rather than being passive biological matrix, tissue is a reactive system, especially in regard to blood flow management, much of it controlled locally and autonomously [12]. A tonometry study of post-surgical patients found that tissue pO_2 could decrease simply as a result of pain stimulae, with vasoconstriction occurring locally through catecholamine increase as part of a regulatory autonomic nervous system response [13]. Unimpeded blood flow is central to the oxygenation status of tissues, and relationships between flow and pO_2 have been the subject of investigation, especially in relation to brain circulation [14]. The state of oxygenation of tissues is a critical determinant of whether the tissues can carry out their normal, energy demanding functions [1]. These range from maintaining membrane exchange processes, undertaking cell renewal, synthesis and various secretory and absorptive processes [1]. Almost immediately with the loss of blood supply to a tissue, or a decrease in delivered pO_2 due to circulatory failure, cell metabolism will shift to anaerobic with lactate production [15]. For the purposes of clinical assessment any pO_2 below 75 mm Hg can be considered to generate hypoxic damage to some degree, hence the importance of measuring pO_2 in biomedicine and physiology.

The supply of oxygen from the arterial circulation through the tissue capillary bed is the effector pathway for O_2 supply, but rapid diffusive transfer through the interstitial tissue space to cells is also important [16]. In certain conditions it could impose a limitation on cellular oxygenation. Work on central nervous system (CNS) tissue using microelectrodes has shown, for example, that there are steep oxygen gradients around small vessels extending over multi-micron scale distances [17]. Ultimately, it is what is in tissue that should be of overriding interest for the understanding of disease. Central blood supply status, i.e. arterial pO_2 , may only give a partial picture for optimal clinical decisions. Pulse oximetry is an important means of tracking changes continuously, especially so since it is non-invasive. However, measurement of haemoglobin saturation, provides an assessment that is localized to the circulation, and only an indirect means of gauging pO_2 [18]; its relation to the latter needs to factor in the uncertainties of a non-linear haemoglobin dissociation curve.

Whatever the intrinsic merit of tissue pO_2 monitoring may be, it is a technically challenging, even experimentally [19,20]. The sample matrix now is non-fluid, complex, less well defined structurally and its solute mass transport retarding effects a matter of conjecture in many cases. Also, it is biologically reactive to a foreign body and capable of changing its composition in order to reject an intrusion. Gough examined the permeability of tissue juxtaposed to a sensor and the later consequences of reactive changes and final device collagen encapsulation [21,22]. Whilst these changes may affect measurement, there is also the early uncertainty of implantation trauma and bleeding leading to measurement errors [23]. This problem of metrology is further confounded by the lack of a reliable reference method for tissue oxygen.

The Clark electrode has been the mainstay of biological and biomedical pO_2 measurement since its invention [24]. Through the simple agency of a hydrophobic, electrolyte impermeable membrane, oxygen is registered within a stable electrolyte film avoiding wider sample matrix contamination and biofouling. Miniaturisation has been challenging due to the need for a hydrophobic O_2 permeable membrane such as of polytetrafluoroethylene (PTFE) or poly(propylene), since seamless bonding to the rest of a device becomes a problem creating material discontinuities and a risk to safe *in vivo* use. At large electrodes PTFE and polypropylene membranes are held in place using retaining structures because of their poor adhesion qualities but this is difficult at needle size electrodes, especially if a coherent liquid film is also to be retained [25,26]. Moreover, taking an internal electrolyte film through sterilisation and protracted storage stages is not feasible. A pre-sterilized tissue pO_2 electrode, amenable to clinical use is needed for clinical oxygenation studies, and of potential value in acute states. If continuous readout becomes possible, as in one example using micro-electrodes [27] new insights can be secured. Also, a pre-selected sensor location could also help focus on particular tissues. Regardless of design, at the fundamental level all such devices when using Au electrodes rely on reproducible flux and a non-passivating reduction of oxygen. Whilst the summary reaction is commonly given as:

in reality it involves a complicated cascade with initial adsorption of hydrated oxygen, complex surface reactions and a reliance on surface catalysis. Unlike anodic reactions in biological samples which are subject to numerous adventitious redox reactions from commonly present organic species, for oxygen there is no competition normally from other reducible species; halothane and nitrous oxide anaesthetic agents are the only exceptions in the clinical situation.

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Because of the implantation need, the classical Clark electrode has seen a variety of modifications [28,29], yet it is challenging to miniaturise. One microfabricated example comprises a 3-electrode arrangement on a glass substrate, with a fluorinated ethylene propylene oxygen-permeable membrane, and a PDMS (Polydimethylsiloxane) reservoir for the internal electrolyte [30], but implantable use would be difficult to envisage with glass. Other polarographic electrodes with electrodes pre-immersed in larger internal electrolyte reservoirs have been described [31]; here oxygen diffuses through a monolithic tubular wall. In a commercial variant a 0.8mm tip diameter polypropylene tube is in use, and designed mainly for CNS monitoring [32,33]. A challenge with an internal electrolyte reservoir is extended diffusion distances and a slower response, though response times have been adequate for clinical purposes. A membrane-free alternative for protecting the sensing surface and establishing diffusion control is the recess electrode format [23,34]. Fabrication may be more complicated, with a particular need to protect the tip during percutaneous insertion.

We have used polyurethane membranes as diffusion barriers for amperometric glucose biosensors [35,36], like many researchers following the landmark paper of Shichiri et al [37] on a glucose needle. We have further found that the physical incorporation of Triton X-100, a neutral surfactant, reduces biofouling and plasticizes the membrane to help manipulate solute permeability [38]. We present an oxygen needle sensor using a polyurethane-Triton X-100 combination membrane, tested for the first time as part for *in vivo* oxygen. The aim was to achieve a biocompatible barrier and a permeable phase allowing electrolyte uptake from the sample, obviating the need for a separate electrolyte film. The electrode materials were amenable to γ -radiation sterilization. We set up a simplified implantation protocol where short-term variations of tissue oxygen were quickly picked up. The sensors were used to monitor healthy volunteers undergoing graded physical exercise.

2. Experimental

2.1. Materials

Phosphate buffer saline tablets (pH 7.4), sulphuric acid (95%), bovine serum albumin (BSA), Triton X-100 (TX) and other standard reagents were obtained from Sigma-Aldrich (UK). All solutions were prepared in deionized water (MilliQ). UV superglue LOCTITE 3211 was purchased from Loctite, Herts, UK. Conductive epoxy was purchased from RS Components (UK), stainless steel

tubes (i.d. 0.38 mm, o.d. 0.50 mm), polyester insulated gold wire (0.125 mm diameter) were obtained from Goodfellow, Cambridge, UK and polishing films (aluminium oxide) 1, 3, 5 μm , were purchased from Thorlabs, LTD, UK. Polyurethane TrixeneSC7602 is from Baxenden Chemicals Ltd, Accrington, UK. A medical grade trocar cannula Vasofix[®] (22 G, 0.9 x 25 mm), was obtained from Braun, Germany. Ultrapure (99%) oxygen and nitrogen were purchased from BOC Group plc, UK. Validation was undertaken using the Oxygen to See (O2C) monitor from Medizintechnik, LEA, Germany (<http://www.lea.de/eng/indexe.html>); this measures blood flow (laser doppler), tissue oxygenation and 'relative haemoglobin', a measure of capillary filling pressure (photo spectrometry). Oxygenation measurement is based on 20W white light (500-800nm) scattering in the target tissue/targeting depth and measurement of reflected light wavelength shift. Measurement is independent of the vascular content of a tissue with changes only monitored in vessels of <100 μm diameter.

2.2. Methods

in vitro measurements were undertaken at room temperature ($22 \pm 2^\circ\text{C}$). Amperometric oxygen measurements were based on a two electrode arrangement using a constant potential of -0.6 V vs. a stainless steel as pseudoreference electrode. This avoided the risk of toxic Ag a reference electrode material, allowed the needle cylinder itself to be part of the electrochemical cell. Consistent with the Clark electrode principle, the nA draw of current meant that a two electrode cell was sufficient and did not cause current polarisation at the pseudoreference. For this system, nevertheless, the plateau oxygen current was at polarising voltages similar to those with a Ag/AgCl electrode. We used a gold working electrode as it is well established for use in the Clark electrode, providing an efficient surface catalysed reaction. A PalmSens potentiostat (Palm Instruments BV, Netherlands), controlled by a PC with associated electrochemistry software (PS Trace) provided for a low intrusion platform for the later *in vivo* studies. A three-electrode set-up (Ag/AgCl reference, platinum counter) was used for cyclic voltammetry with a standard laboratory electrochemical analyser (CH Instruments Inc.).

2.2.1. Electrode calibration

For calibration, 50 ml PBS was purged of oxygen using continuous bubbling with nitrogen for 30 min with the electrode was poised at -0.6 V vs the pseudo reference. Current was registered in the oxygen free solution at 25°C .

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A PBS solution saturated with oxygen using continuous oxygen bubbling was used to determine the 100% response, respectively. Under a nitrogen atmosphere, the electrode was immersed in 10 ml unstirred oxygen purged PBS, and aliquots of 1 ml PBS at 100% O₂ saturation injected. Ten repeat injections provided a series of oxygen levels and a family of plateau current values to allow derivation of calibration plots.

2.2.2. Sensor construction

The needle construct was similar to that previously used for lactate monitoring [39], except that the working electrode material was an insulated gold wire not platinum wire. The sensing element followed the Clark electrode principle where gas membrane is combined with an internal electrolyte and a non-adherent membrane, but that is not adherent with the difference that our sensor used body fluid as electrolyte by avoiding a gas permeable membrane. As with the Clark electrode a low current flow (nA) ensures a stable polarisation voltage. There are no reducing interferents in solution, so we did not need another membrane. The multilayer deposition of polyurethane provided a diffusion limiting barrier, so it was unaffected by external diffusion limitation constraints. The polyurethane here provided a microporous diffusion limiting membrane.

A polyurethane layer [40] was coated onto the sensing tip using polyurethane pre-polymer solution prepared by dissolving 20% (w/w) Trixene SC7602 (the kind gift of Baxenden Chemical Ltd UK) in tetrahydrofuran. The solution was drop cast over the tip of an inverted needle and dried overnight under ambient conditions at room temperature ($25 \pm 2^\circ\text{C}$) to ensure polymer adhesion to the needle shaft and completion of cure. When surfactant was employed, it was pre-added to the Trixene/tetrahydrofuran solution; sensors as prepared were used for *in vitro* studies. For *in vivo* use, sensors were washed in 70% (v/v) ethanol and ultrapure water for 30 min, and packed in sterile plastic bags (Westfield Medical, UK) kept sealed throughout the application of the sterilisation protocol until removal for *in vivo* insertion. For animal studies, sensors were sterilised with 70% ethanol following opening of the sterile storage bags.

2.2.3. Sterilization protocol

Sensors for human studies were sterilised by γ -irradiation, using a medically compliant cold sterilisation process for implantable devices; simpler autoclaving was not a feasible option. Bactericidal action here is based on energetic biodisruption due to the extreme high energy and

frequency of γ -rays. Four methods, centered on microbial bioburden analysis, as assessed by bacterial culture, are used to define a device as being sterile according to ISO11137 2:2007. These also establish the minimum acceptable dosage that may be permitted for a device to be certified as sterile. The choices available for sensor sterilization were the following: Method 1, which stipulates the specific minimum radiation dose needed for a device, using bioburden assessment, to establish an exact dosage requirement at 12.6kGy. This would require large sample numbers: 130 sensors. Method 2 is a last resort protocol for the absolute minimisation of radiation damage used for sensitive devices, allowing tailoring of dosages at under 10kGy. This is costly and time consuming, requiring industrial scale sample volumes: 840 sensors. Methods 3 and 4 are known as as VD Max (Verification Dose Maximum) and use pre-set radiation dose at either 15kGy or 25kGy. These apply a pre-defined minimum dose for sterility. The advantage here is that exploratory dose tailoring is not needed, and sample numbers are relatively low, though even here, testing requires 40 sensors at the final specification which are then unusable. A VD Max regimen was selected here. For VD Max at 15kGy, a device needs to achieve CFUs (Colony Forming Units - organism number) down to <1.5 CFU and for 25kGy CFU down to only <1000 CFU is necessary. So to minimise labour-intensive sensor construction and to comply with the lower tolerance CFU, VD max at 25kGy was used. For this batched samples were fabricated and sterilization commissioned with a certified provider (Isotron Ltd, UK).

2.2.4. *in vivo* studies

Two male Sprague Dawley rats (380g and 450g) were anaesthetised using an isoflurane (2-chloro-2-(difluoromethoxy)-1, 1,1-trifluoro-ethane (Isoflurane)/oxygen mixture in accordance with a licensed UK Home Office procedure. Once the rats were under full anaesthesia their backs were shaven in order to facilitate subcutaneous implantation of the sensors. Sensors were implanted in the dorsal region to a depth of about 3 mm using a hollow needle as an initial insertion guide for the electrode. This study was intended to demonstrate dynamic respiratory oxygen level tissue effects and their recovery within an individual animal; a control animal with a different physiological background is not relevant to a sensor evaluation such as this.

in vivo monitoring was undertaken on eight healthy male volunteers (there was no gender restriction) in accordance with the protocol approved by the SE London Regional Health Device Ethical Committee. As with the rat study, controls were not used as the study was a first in practice

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evaluation of a new sensor for human monitoring, not a study of physiology per se.

Sensors were *in vitro* calibrated using sterile solution and then implanted using a standard clinical commercial trocar – plastic cannula set, received in pre-packed sterilized condition. Insertion of this cannula combination into subcutaneous tissue was at $40 \pm 10^\circ$ to a cylinder length of up to 2 cm. After insertion, the trocar was withdrawn from the cannula which remained in place. This procedure enabled disruption-free advancement of the sensor tip, avoiding tissue and membrane damage or any bleeding outcome immediately around the end of the needle. In the procedure, the needle was slowly advanced through a tailored, pre-cut, cannula designed to allow a 1.5 mm length of the needle to project from the tip into tissue (Fig 1). The external entry point and remaining electrode shaft were then enveloped in protective surgical plaster to stabilize the device, reduce angular motion in tissue and maintain the original insertion angle. Electrodes were implanted either in the forearm or the dorsum of the hand and monitoring undertaken over short periods during which some undertook periods of intense exercise of graded severity; for this, a bicycle ergometer was used. The hand exercise enabled monitoring of tissue oxygenation at a localised exercising muscle mass with its own segmented arterial supply and venous drainage. The cycle exercise, however, was a whole-body exercise with monitoring away from the active muscle mass, with ergonomic control allowing for equivalent, controlled, exercise loads across the subject group. In this way local vs remote tissue oxygenation change was compared. The ergonomic bicycle has become an established tool in sports medicine and athlete preparation. For practical purposes, these forms of exercise also allowed us to monitor high activity under static conditions without the need for wireless monitoring. Post implantation recalibration enabled assessment of biofouling and the validity of the tissue measurements.

3. Results and Discussion

Oxygen was measured on the basis of reductive currents at an Au cathode. An equivalence of response to pO_2 , in stirred and unstirred solution was checked. Independence from stirring in this way provided a short form check on the capacity of the sensor to generate a stable response in tissue, independent of tissue biological variation. The polyurethane barrier was key to this and needed to be diffusion limiting yet thin enough to avoid slow response. Reliable operation of such devices is entirely dependent on a diffusion control element definitively residing in the sensor and not the surrounding sample matrix [21,22]. We have previously investigated PTFE, silicones and

polyurethanes for oxygen selectivity and permeability [40,41] and for resistance to biofouling. Polyurethane (Trixene SC7602) was chosen here, as it conferred the desired linear response over the physiological range (0 - 160 mmHg pO_2), the level of permeability needed for electrolyte access from the sample and relatively short response times; 5 min for a plateau response. Without the surfactant, response times were longer, eg >10min. A systematic comparison was not undertaken of response times here; our early work showed increased solute permeability through use of surfactant [38]. The primary value of the surfactant here was its antifouling action to limit electrode drift; a comparison was not undertaken, but the absence of drift in albumin solution and low drift *in vivo* is unattainable with polyurethane alone; our prior work [38] established this at an early stage.

Ascorbic acid as the most significant electroactive component in biological fluids had no interferent effect at cathode potential, but nor did it passivate the electrode during continuous operation. Addition of ascorbate to 5mM in a buffer solution in an ambient atmosphere gave no additional response. Biofouling was a potential problem, with albumin the dominant protein in plasma and in interstitial fluid (ISF); the first to arrive at a surface before its partial competitive removal by other proteins [42]. Triton X-100 incorporated into the membrane using 2% w/w in the casting solution reduced fouling due to albumin (40mg/mL in PBS), tested to 17h (Table 1). Surface contamination was not completely avoided on the basis of response, but stability was adequate for *in vivo* studies. Without a membrane, bare electrode response decreases by 40% under the same conditions [41]. Figure 2 shows the oxygen calibration curve for the physiological range 0 to 160 mm Hg measured in a phosphate buffer saline (PBS) and 40 mg/ml BSA. Though a small drop in sensitivity is observed in BSA, this was not cumulative and measurements in each solution gave linear and repeatable responses. There was no stir dependence and no drift during the calibration periods. Figure 3 shows *in vivo* changes in an anesthetized rat. The time trace shows a sensor stabilisation period of 1 h before the current reached a baseline; this was low, however, and equated to a pO_2 of only 15 mmHg. A small perturbation at 20 min coincides with the point where oxygen supply was changed, and flow stopped for 2 min; after this O_2 flow was returned to 2 mL/min. Once a stable baseline was reached, oxygen flow was increased to 4 mL/min which rapidly increased pO_2 , an effect reversed by return to 2 mL/min and reduction of oxygen to only 15 mmHg when 1 mL/min was used. Later increment to 4 mL/min re-established the higher pO_2 prior to termination of the monitoring period. Electrode changes were registered within 1min of an oxygen flow change.

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Monitoring of a human subject is shown in Figure 2C. The sensor here was forearm implanted in a sitting volunteer. Venous and arterial occlusion to blood flow to the arm was carried out using a manual sphygmomanometer. The sensor was first allowed to stabilize over 13 min. During this period rapid, 30s, low amplitude pO_2 fluctuations were observed, and in the 100s venous occlusion phase, these pO_2 oscillations were maintained but with no obvious pO_2 trend. The 120s arterial occlusion phase led to the disappearance of the oscillations and a steady decline in pO_2 was evident until the cuff was released when a rebound increase in pO_2 was seen. The periods of occlusion were kept to a minimum to reduce discomfort to the subject whilst allowing demonstration of a trend in pO_2 . Sensor response was immediate to any arterial occlusion. Occlusion outcomes based on forearm tissue

pO_2 have been reported previously with a transcutaneous heated skin electrode, but a continuous readout being shown [43]. Here, a steep exponential decay in pO_2 to near zero values was seen within the 7 min, a contrast to the present slow, linear reduction. Methodological differences may have been responsible; a large surface area transcutaneous electrode would have been a significant sink for oxygen and heated skin permeability to oxygen through adult skin will have restricted flux. Thinner neonatal skin enables greater oxygen flux, and continuous readout here has shown some evidence of oscillatory oxygen behaviour [44] at a periodicity of around 1-2min. Higher frequency oscillations, such as in this study, however, were reported using an injected phosphorescent reagent loaded gel; this functioned through oxygen optical quenching [45]. Here also, O_2 levels approached extreme low value, remarkably within only a minute. This suggests a local supply issue; such convergence to anoxia would seem to be difficult given the likely availability of a residual oxygen reservoir in a non-exercising arm. Monitored changes during bicycle exercises in another subject are shown in Figure 2D. The sensor was dorsal forearm implanted. Stabilisation to baseline oxygen at 15 min indicated that tissue pO_2 in this subject was ~35 mmHg, much higher than in the rat and comparable to that in another human study [6]. Exercise severity was ramped up using increased cycling speed, not ergometer load. When the free cycling period commenced, a rapid rise in pO_2 was induced, and with a more marked increase to 80mmHg oxygen after the subject cycled to their maximum capacity. As the volunteer became fatigued, cycling speed dropped, followed by an immediate drop in pO_2 . A further increase in cycling speed at 35 min caused pO_2 to finally rise, with a later end reduction as cycling ceased, with pO_2 returning to baseline level. During each cycling period, a fall rather than a rise in pO_2 would be expected on the basis of increased oxygen deficit. For another subject (Figure 6), baseline pO_2 was similar (45 mmHg). Cycling load was now achieved through

ergonomic resistance, and again led to increased pO_2 to 75 mmHg, with 110 mmHg achieved after doubling of the load, the upper end of normal for arterial blood pO_2 .

The third subject had a much lower starting pO_2 (Fig 3B) but, interestingly here the electrode was implanted in the back, and there may be locational differences as well as any inter-individual differences. showed the same graded exercise increments in pO_2 . The small trough in pO_2 trough at 7min coincides exactly with a dip in cycling effort. Forearm clenching exercise conducted in one seated subject was preceeded by a 10 min arterial occlusion which showed a slow limited slope decay in pO_2 (Fig 4A), again a contrast to reported, rapid approach to anoxic tissue conditions [44,45]. Fist clenching and relaxation was undertaken over varying periods with the sensor implanted in the exercise forearm. Again, there was a rise and not a fall in pO_2 , but it was much steeper than during whole body exercise. The same observations were made in a second subject (Fig 4B). Sensors for all these studies were recalibrated after retrieval, and in each case drift due to any fouling was not significant (<4%) in the context of the changes seen during monitoring. There may have been drift in the tissue due to a cumulative foreign body response around the device, but this seems unlikely to have affected the monitoring given the prolonged period needed for the accumulation of inflammatory tissue and later fibrosis. As with all such sensors, movement artefact could be induced at implanted sensors, but this was mitigated by their residence in a cannula and avoidance of physical motion during the sedentary exercises. For free moving monitoring, movement artefact is a highly likely challenge, but since the oscillatory signals this generates have periodicities of seconds, noise suppression routines are likely to overcome the problem. Indeed, this is a likely approach used for in vivo glucose sensors allowing their use in ambulatory patients as a norm. Exercise leads to high levels of oxygen consumption by muscle with an associated surge in lactate. The measured oxygenation state, a balance between local O_2 vascular delivery and tissue demand has been the subject of few studies [46,47] other than for brain oxygenation monitoring. In the present subjects, regardless of exercise status, there were constant fluctuations in pO_2 involving a mix of rapid and slow changes of different amplitudes but nearly all <15mmHg. The amplitude observed is well above the analytical in vitro signal to noise seen with these electrodes (<1%) and are therefore not artefactual or random noise. As well as the microelectrode, a further basis for noise suppression is the barrier membrane which dampens the hydrodynamic noise typically seen at uncovered electrodes, albeit at the price of a slower response. Patterns showed some individual characteristics; in two subjects there were only low level small fluctuations (Figs 3B, 4B) of 10-15s and 1mmHg amplitude. Rapid blood flow fluctuation is seen together with its representation as

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O₂ oscillations though such observations are typically not remarked upon [48]. In contrast to the larger undulations, these could be neglected as biological or electrical noise, but the possibility of flow variation, reactive or otherwise, in the microcirculation around the sensor cannot be ruled out. Much depends on the speed and sensitivity of the electrode response; a slower electrode response is less likely to allow for these observations. The oxygen sensor used in these studies had relatively rapid, response times [8]. The larger time scale tissue oscillations of pO₂ have certainly been seen and remarked upon in previous animal studies. They have been observed in experimental animal models, over extended implantation time scales [21] and during acute study of muscle and cancer tissue [49,50] where high and low frequency changes were evident when recess tip microelectrodes of very fast response (<0.1s) were used. The cause of these changes will have been spontaneous shifts in blood flow through arteriolar vasomotion. Oscillatory frequencies reported are wide ranging but have a reported frequency dominance between 8 – 60 cycles/h. This is the first study reporting rapid fluctuations in human subjects. The findings are also consistent with evidence for rhythmic oscillations of the small blood vessels supplying tissue [51,52]. Theoretical models also support the proposition of oscillatory input into tissue delivery [53]. There is a wider potential implication for the movement of intermediary metabolites. In the case of the tissue implanted glucose biosensors, oscillatory glucose behavior has been observed in an animal model at frequencies of 2-8 cycles/h; this is suppressed by general anaesthesia [54]. There is a possibility that some of this effect might be due to background pO₂ fluctuations affecting glucose oxidase enzyme activity in the glucose biosensor so comparisons with non-oxygen dependent sensors would be of value. As well as inter-individual differences suggested by our work here, there may be interspecies differences; in the rat experiment, the oscillations were much finer amplitude, though the animal was under different conditions - anaesthesia (Fig 2B). The increase in subcutaneous tissue pO₂ during exercise (Figs 2(A,B,C,D), 3(A,B)) is as yet an unreported phenomenon. A strong possibility for it is increased blood flow towards the skin in competition with muscle during exercise due to the overriding need for thermoregulatory control and disposal of heat from the body. At rest, skin takes only 5-10% of cardiac output but during exercise this can increase 5-fold [55]. With increased blood flow and unchanged metabolic requirements pO₂ would be expected to increase. pO₂ elevation during forearm only exercise is more difficult to explain (Figs 4A,B), however there is a marked associated increase in forearm blood flow during such localized exercise [56] which may lead to oxygen delivery to skin. pO₂ measurements on muscle can be confounded by the elevated oxygen saturation due to such increased superficial skin flow [57]. In a hand grip exercise study, it

was found also that local, rather than general, vasodilatation occurs [58], apparently mediated by muscarinic receptors. Whilst oxygen levels decreased abruptly during arm exercise in the current study, post-exercise hyperaemia with increased skin flow in the vicinity of exercising muscle might be expected [59] but was not seen in this study. A possible contribution to the rise in electrochemically measured pO₂ is a Faradic temperature effect on the electrode. As a Faradic process there will be a temperature dependence of the electrochemical reaction, but since this is a diffusion control system this will operate via the temperature dependence of the O₂ diffusion coefficient, assuming that O₂ membrane partitioning is constant. Skin temperature is a variable but will only be a small factor. Minor rise was seen in one study generating a temperature range of 31–34°C irrespective of exercise intensity [60]. These are similar values to the range seen at rest (32–34°C [61]). The more general understanding of this physiology, however, is that temperature goes down in exercise [62]. The possibility of a flux dependence, ie oxygen delivery dependence, of the needle sensor response remains, despite a diffusion limiting membrane. This would make the device blood flow dependent, and simply lead to tracking of blood flow not true local pO₂. Further studies are required to resolve this possibility, but it exists for all implantable amperometric sensors. A comparative analysis with non-invasive pulse oximetry of SO₂ under conditions of rapid intermittent sphygmomanometer blood flow occlusion are, however, reassuring on this point. Oximetry is flow independent and measures a different modality, but despite this it shows a matching trend (Fig 5). There is also a very similar rapid oscillation in oxygen/oxygenation levels. Some biological reasons for a difference would also seem likely; different body compartments were being monitored, respectively intravascular and tissue, and the tissue sampling zone for the electrode being would be more superficial than with O₂C. Nevertheless, the drop in oxygen is steep, abrupt for both but with a lag effect of 2-3 min before any change is registered. This may be due to the reservoir of available residual oxygen in the limb. This dual monitoring constitutes one of the few correlations of tissue oxygen with a comparator measurement. Irrespective of quantitative uncertainties in the absence of a reference method, it reinforces the possibility of electrode based trend monitoring of pO₂.

4. Conclusion

A needle based amperometric sensor for oxygen monitoring in tissue was tested *in vitro* and *in vivo*. The data from the sensors were validated with commercial oximeter. The sensors had a linear output covering the physiological oxygen range and were significantly

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resistant to biofouling. pO_2 oscillatory patterns were observed with and without exercise which were consistent with those reported for vasomotion, but this has been the first such study in humans. The cutaneous hyperoxia response to systemic exercise appears to be determined by the requirements of thermoregulation and could possibly provide an independent index of exercise tolerance. Future work with rapid response sensors characterising oscillatory behavior will be able to provide new leads into pathological circulatory changes, for example in peripheral vascular disease and in diabetes where vasoactivity may well be altered. Extension to fast response sensors for metabolites more widely will add parallel insights. Whilst studies are beginning to establish a capability for practical tissue monitoring based on a blood equivalence. Instead, tissue should be considered an independent window into the body, and faster response sensors, giving information on vasoactivity effects on metabolism are likely to be of added value. What the pioneering experimentalists have taught us could in future be extended to the clinical environment. Oxygen was measured on the basis of reductive currents at an Au cathode. An equivalence of response to pO_2 , in

into subcutaneous tissue of the forearm. Oxygen measurement was undertaken over 25 min by application of -0.65 V vs. stainless steel pseudo reference electrode with a battery operated potentiostat. Venous (V) and arterial (A) occlusion, indicated by the rectangles (enlarged below) was generated using a sphygmomanometer. With venous occlusion, no oxygen trend was seen, and the baseline oscillations continued, however, with arterial occlusion, there was a downward trend in oxygen and the oscillations were dampened (C); Human *in vivo* study: subject undertook cycling exercise on a load free ergometer with needle oxygen sensor implanted in the subcutaneous tissue in the dorsum of the forearm (the measurement conditions are as described for Fig. 2C); “up” indicates cycling at maximum speed, “down” at lower speed (D).

Fig.3. Human *in vivo* study: subject cycled on an ergometer with an oxygen sensor implanted in the dorsum of the forearm. The measurement conditions were the same as described in Fig. 2C, but different cycling loads were applied to vary exercise severity (A); Human *in vivo* trial – a volunteer was cycled on an ergometer with an oxygen sensor implanted in the subcutaneous tissue in dorsum (exercise conditions as in Fig. 2C) (B)

Fig 4 Human *in vivo* trial –arterial occlusions using sphygmomanometer and after that the volunteer was clenching his fist with an oxygen sensor implanted into the subcutaneous tissue of the forearm (the measurement conditions were the same as described in Fig. 2C) (A); Human *in vivo* trial – a volunteer was clenching his fist with an oxygen sensor implanted into the subcutaneous tissue of the forearm (the measurement conditions as described in Fig. 2B) (B)

Fig. 5. Intermittent application of a forearm sphygmomanometer to achieve arterial (A) and venous (V) occlusion using a needle sensor () and a commercial oximeter instrument ().

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Figure captions

Fig 1 A) Schematic of the oxygen sensor showing longitudinal section with the core working electrode of diameter $125\ \mu\text{m}$ housed in a stainless steel needle. B) Image of oxygen needle sensor housed in a cannula prior to insertion.

Fig 2 Calibration curve of oxygen sensor in PBS, pH 7.4 () and in $40\ \text{mg/mL}$ BSA/PBS, pH 7.4 (). Measurements were undertaken at a constant polarising potential (-0.65 V vs. stainless steel) in the physiological range (A); *in vivo* testing of an oxygen sensor in a Sprague Dawley rat ($450\ \text{g}$). The sensor was inserted in the dorsal part of the anaesthetised rat, which was breathing a mixture of isoflurine and oxygen throughout the experiment. The body temperature was maintained at 37°C with a thermal blanket. A constant potential of -0.65 V vs. a stainless steel pseudo reference electrode was applied to measure oxygen level in tissue. The changes in tissue oxygenation were generated by changing to the oxygen flow in the breathing mixture. L – low, $1\ \text{mL/min}$, N – normal, $2\ \text{mL/min}$, H – high, $4\ \text{mL/min}$. C – denotes O_2 min gap for a change in the O_2 cylinder (B); An oxygen sensor was inserted at 45°C

Table

Influence of surfactant on early and extended period biofouling of oxygen sensor

Notes: The authors declare no conflict of interest.

Data Availability statement: The data that support the findings of this study are available from the corresponding author upon request.

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