Monitoring with *In vivo* electrochemical sensors: navigating the complexities of blood and tissue reactivity

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Abstract

The disruptive action of an acute or critical illness disease is frequently manifest through rapid biochemical changes which may require continuous monitoring. Within these changes, resides trend information of predictive value, as well as responsiveness to therapy. In contrast to physical variables, biochemical parameters monitored on a continuous basis are a largely untapped resource because of the lack of clinically appropriate monitoring systems. This is despite a huge testing repertoire opening up in recent years in relation to discrete biochemical measurement. Electrochemical sensors offer one of the few routes to obtaining continuous readout and, moreover, as implantable devices information referable to a specific tissue location. This review focuses on new biological insights that have been secured through *in vivo* electrochemical sensors. In addition, the challenges of operating in a reactive, biological, sample matrix are highlighted. Specific attention is given to the choreographed host rejection response as evidenced in both blood and tissue and how this both limits sensor life time and the reliability of its operation. Examples will be based around ion, O_2, glucose and lactate sensors because the importance of this group in acute health care.

Key words: metabolite sensors, sensor biocompatibility, ion selective electrodes. Foreign body reaction. O_2, glucose, lactate.

1. Introduction

Physiological processes operate under highly dynamic conditions conditioned by a multitude biofeedback systems operating on both long and short term timescales. These establish a homeostatic control within finite set limits. It is the essence of any multi-cell and tissue organism that it is able to maintain relative internal stability in the face of unpredictable, and often undesired, environmental change. Within the cell itself, sensitive surveillance mechanisms recognise status deviation and effect timely responses, on an ultrafast basis if deemed necessary. These responses are all the more effective when delivered through specialist tissues and the major organs. Complexities at cell level are only partially reflected in changes in the extracellular space. However, it is only the changes here that we are able to monitor and assess through available sampling and measurement capabilities. For some extracellular parameters, these changes take place on compressed time scales and justify frequent, if not continuous, measurement for both a better understanding and for the management of with disease. These variables can be considered labile and their dysregulation represents a major failure of homeostatic control. Currently, these centre on ions, gases and small metabolites. Their potential for undergoing rapid change is also indicative of the potential value of continuous monitoring to track their trajectory and to help titrate therapy. The timing of any therapy is of equal importance to its amount in certain types of critical illness, and may influence recovery and survival.

For all practical purposes, only extracellular events are trackable. Moreover, the fractal and multi-organelle architecture of the cell does not readily offer simplified messages that allows easy conclusions about its status or that of the whole individual organism. This remains the case even if robust microsensors were to become available. A clear differentiation between normal and disease is vital clinically, and whatever the biological value of intracellular monitoring, any information thus secured comprises a complicated case mix affected by cell ageing, the cell cycle, compartment sampled, the reaction of the cell and a myriad of other unknowns that are not easily synthesised into workable diagnostic formulations. The occasional exception is where disease disruption is so overwhelming that it leaves a substantial intracellular signature bordering on structural change such as or when an insoluble end product accumulates or a core metabolic process is involved as in some inherited metabolic diseases.

The earliest effort with sensors for was indeed directed at intracellular monitoring, but for enhancing biological understanding. This involved pioneering work with wire and micropipette electrodes. The latter as ion selective electrodes were particularly prominent, and were used to variously follow intracellular H^+, K^+ and Na^+, in part to study cell plasma membrane exchanges. A particular interest was excitable tissues such as nerve and muscle [1] where ultra-rapid ionic exchanges mediate membrane depolarisation and launch action potentials. Such work initiated our understanding of cell and physiology and its ability to function at high speed. Oxygen was the other intracellular target, enabled through more robust wire-based electrochemical sensors [2]. Priority interest in oxygen emanated from, and remains, its central importance to the energy economy of the cell and the dire consequences of its deficiency.

The cell, tissue and whole organism hierarchy gives us a dimensional scale across which we can select monitoring options at the supra-cellular level. For clinical purposes, it is at the

whole organism end of the range that we can see direct clinical value. This larger scale is fortunately readily accessible to us via the circulation, accommodating invasive probes. Whole body physiology targeted this vascular access to understand change in the intact organism. The current medicine paradigm is the use of blood as the ultimate pre-mixed representative of whole-body change. Whilst blood is core to our disease understanding, it is still an approximate messenger, affected differentially by different tissues and with a compositional change that may also be a diluted version of events at local level. Our access and assay technologies in the clinical laboratory are inevitably directed at blood. A typical biochemistry repertoire is 200 parameters, a triumph for analytical science [3], but one less attentive to tracking rapid change other than by more discrete measurements. Yet we already know the value of continuously monitoring oxygen, certain ions and metabolites in blood.

Other fluids such as urine and CSF might have discrete measurement value, but continuous monitoring is unlikely to be of additional benefit. The tissue biopsy [4] for targeted analysis lies at the extreme end of the scale for any repeat measurement need. The developing blood biochemistry repertoire has made it possible to gain a better picture of the timescale for variation. Homeostatic control, far from creating a biochemically static environment, effects a pattern of pre-set cycles and modulations. Chronobiologists have elucidated such patterns for the healthy state, segregated variously into ultradian, circadian and other longer-term and less well understood forms of biorhythm [5]. In disease, there is not only a deviation from set points for a biochemical parameter, but moment to moment fluctuation which may change in character and contain added information. This goes unrecognised because continuous tracking is not available, and the nominally trivial. Rapid change possibilities were understood in relation to monitored blood gasses: pH, pO_2, pCO_2 [6,7]. However, from what we now know of glucose and lactate, these and other intermediate metabolites may well exhibit rapid change.

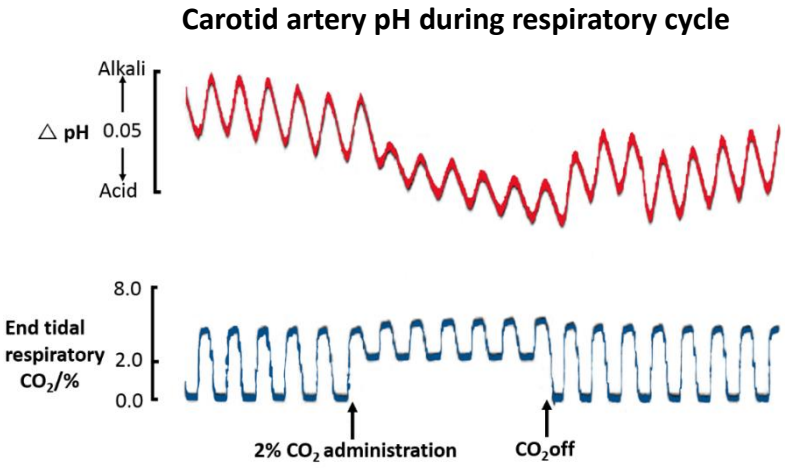

Fig 1 Arterial blood pH monitored extracorporeally at a carotid artery loop in an anaesthetised cat using a glass pH electrode. End tidal CO_2 was monitored by an infrared CO_2 analyser. Administration of 2% CO_2 led to increased end tidal CO_2 and a drop in arterial pH. The pH trace also shows breath to breath arterial pH oscillations. Adapted from [12].

In examples relating to ions, continuous monitoring of blood was undertaken in animals using ion selective electrodes (ISEs) in early physiology studies on animals [8-11]. Striking rates of change were seen. These studies were remarkable in being well before the advent of the

microfabrication toolkit for reproducible miniaturisation. In one example, rapid change to arterial blood pH was shown a result of increased inspired CO_2 content (Fig 1) [12]. Conversion of CO_2 to carbonic acid by carbonic anhydrase in red cells, we know, is rapid, but its manifestation as a matched fast outcome seen through blood [H^+] change is a useful dynamic indicator. More remarkable, perhaps, was the observation, as in this study, of breath to breath pH oscillation due simply to breath to breath pulmonary tidal pCO_2 variation. The oscillation is a stable, and remarkable for being observable in the highly buffered entity that is whole blood. Interestingly, the amplitude of the oscillations also parallels the respiratory CO_2 excursions. The nature of oscillations in respiratory illness and compromised blood buffering remain an unknown, but could provide new clinically relevant information. However, only a fast response ISE with its millisecond response has the capability of unmask such hidden variables. Sensor advances, combined with signal processing, might provide a valuable step forward in extracting and processing such hidden information. Technically, the active membrane components used in ISEs have changed little, a tribute to the early chemists. However, other new design iterations could be usefully pursued. An ion selective membrane for bicarbonate ion has yet to be made, yet bicarbonate ion has a huge importance for the acid-base status of the body in disease, and its dynamic variation unknown.

A classical basis for the *in vivo* sensor is its ability to follow a trend and thereby pick up deviation early, whilst a parameter remains within the bounds of normality. With wider deployment of such *in vivo* sensors, there will be greater identification of early variation and of hidden dynamic patterns linked to disease. Minor oscillatory and minimal changes will need high resolution sensing. Rapid response will also be crucial, as shown with pH. On speed of response, however, it is probable that slower biosensor tracking of metabolic fluctuations will still be useful. Oscillatory cycles for basal insulin and glucagon have been observed in blood, and are relatively slow, on timescales of minutes. They also have a synchrony with glucose oscillations of similar periodicity [13], though this glucose cycling has an amplitude of merely 0.05 mM. Future sensors able to track such minor cycling behaviour could allow linkage to be made to metabolite control and its dysregulation;

Dynamics of oxygen change at the tissue level were the focus of some early studies. These helped to unravel interrelationships between oxygen delivery centrally and localised tissue oxygen distribution. The latter is modulated by local microcirculation, itself in a state of dynamic flux. Silver [14,15] investigated normal and solid tumour tissue pO_2 using microelectrodes; Lubbers [16,17] used multi-wire electrodes and tracked both oxygenation variability and microenvironment heterogeneity. Time dependent shunting of O_2 was regularly seen, with complex cycling, that was topologically connected to the structure of the vascular bed. The interstitial tissue space was thus confirmed, for oxygen at least, as a seat of complex gradients; here these reflected a balance of cell respiration against the undulations in local blood supply.

A physiology study may only require a sensor to operate for a limited period, and this also under highly supervised, conditions. Progression to medical use poses a more severe challenge where simplified, robust operation is key. Technical refinements and miniaturisation have moved the field along in analytical chemistry terms, but stable *in vivo* embedding and communication has proved a more protracted challenge. In the absence of reference methods, monitored output is always a combination of true metabolic readout and

uncertain, artefactual change due to implant site tissue changes, including those that cause fouling on the device sensing surface. The balance may well be towards a meaningful readout and not artefact, but the latter can prove remarkable mimic of real change. The communication interface with tissue or blood is the weak link. Interface stability is under constant threat from an assembled biological reactivity that changes the very environment intended as a sampling window into whole body change. Our understanding of the complexity of both the process and it structural end products is still limited. Innumerable sensor design approaches, including of new transduction routes, have been reported to combat the adverse effects in the host environment, but success has been partial. For tissue, especially, the sensor is merely another foreign intrusion, and it is of no consequential difference to the biology as to whether it has been architectured as an 'intelligent' material or some standard unreactive biomaterial. Emphasis is needed on the biology now to find a route to reliable monitoring and the promise it brings. Recent work has begun to consider these extra-sensor processes and their key elements. The balance of work, though, still does not reflect the centrality of the biological question. Biomaterials research certainly provide a guide, but sensing research needs its own approach now. On this aspect, the review summarises the response basics in blood and tissue, and how these can affect sensor performance. Examples of dynamic monitoring, however, show why the effort is worthwhile.

2. Sensors for continuous intravascular monitoring

2.1 Ion selective electrodes

The ISE has two intrinsic advantages for monitoring. Firstly, it responds on the basis of facile ion binding and not slow diffusive access to deeper structures. This delivers a response time within milliseconds. Secondly, the response is on the basis of equilibrium ion binding, so a maintained ion flux to the device at steady state is not needed. The first allows unmasking of rapid transients (Fig 1), the second is far less affected by surface fouling, other than possibly by aa slower readout or if the deposited layer itself has charge effects which alter membrane potential. ISE biological use has thus been mostly without modification to *in vitro* device membranes. Indeed, a material as unpromising and as bioincompatible as a glass pH membrane is usable in biological fluids in the first place because of its independence from continued ion flux for stable readings. The more central issue for ISEs *in vivo*, though, is the potential toxicity of incorporated ionophores and plasticisers. Though quantitatively small, they are biologically active and toxicologically risky; the standard plasticiser 2-nitrophenyl octyl ether, for example is pro-inflammatory and ionophores can have cell membrane effects.

In the main, *in vivo* studies have focussed on K^+. It is of predominantly cellular origin, and its abnormal release can both reflect and cause alter the stability of instability in excitable neuromuscular tissue. In an early animal study, Hill et al [18] devised a flexible intravascular catheter sensor for K^+ with a membrane comprising the K^+ ionophore valinomycin in PVC and achieved low drift monitoring with catheter mechanical compliance matched to intravascular use. A tip diameter of 0.6mm enabled safe small vessel insertion without flow obstruction. Femoral vein catheterisation in humans was achieved with a low drift (<3mV/h) catheter which enabled monitoring of rapid K^+ efflux from muscle during exercise; K^+ transients of <0.1 mmol/L were picked up [19]. The pressure artery pose a further catheterisation risk, but arterial catheterisation has also been reported. In an animal study using a carotid artery catheter, rapid K^+ release from carotid body oxygen chemoreceptors was monitored during hypoxia stimulation [20]. The benefits of a precise localisation of a sensor tip was shown using

a catheter advanced into the coronary sinus of the heat in human studies. This vessel serves as the common venous conduit for blood from the heart. In patients suffering from myocardial ischaemia, K^+ transients were shown which would have been undetectable within the general circulation [21]. K^+ release from myocardium, moreover, is correlated with the severity of ischemia [22].

Rapid blood Ca^{2+} transients have also been shown using indwelling vascular catheters. In one report, a synthetic Ca^{2+} ionophore was used to functionalise a plasticised PVC membrane [8]. In a study on dogs, cardiac venous drainage showed Ca^{2+} perturbation due to the injection of ionised X-Ray contrast agent [23]. With glass being unacceptable for *in vivo* use, pH catheters have been made using H^+ affinity polymer and H^+ ionophore, octadecyl isonicotinate [24]. When applied to coronary sinus monitoring, blood pH change could be tracked in cardiac ischaemia patients [25].

2.2 ISE biocompatibility

The active ionophore of an ISE is typically a high affinity, high reversibility binding agent, and if diffusible could pose a risk *in vivo.* The direct consequence is permeabilization of the cell plasma membrane. The quantities used for small *in vivo* ISE membranes are insufficient for systemic toxicity, but could still pose a local tissue risk; regulatory approval I any case would necessitates extensive testing. Discovery research for new ISE membranes may well extend our analytical reach for ions, but now should preferably combine biological with chemical screening. Cánovas et al [26] undertook such combined evaluator studies of ISE membrane components and tested cytotoxicity *in vitro*, notably for valinomycin, the most efficient K^+ ISE ionophore to date. Inevitable toxicity, and the antibiotic mutacin, a polycyclic peptide, was suggested as a possible alternative ionophore for K^+. Much, of course, depends on how much of an ionophore will leach out, and this in turn will be a function of membrane permeabilization due to co-entrapped plasticiser. The plasticisers assessed in this study showed varying degrees of toxicity, and reinforce the principle of pre-use testing. The polymeric component of the ISE membrane should not be a toxicity concern. A polymer is only really toxic in so far as it releases small molecule constituents. This could possibly arise from biological degradation, as seen with polymeric biomaterials. The reactive implant site has high degradation potential with its constituent hydrolytic enzymes, lowered local pH and free radicals release from activated phagocytic cells. So again, biological screening needs to be part of any new polymer development, other than in the case of established PVC or polyurethane.

ISE surface modifications and coatings for safe retention of diffusible components is unlikely, given the parallel need for ion permeability. However, the possibility exists for reducing surface biofouling by a coating. Pharmacologically active agents for stabilising blood platelets at the surface could help mitigate fouling, and surface hydrophilic layers such as of polydopamine [27] and hydrogels such as poly(2-hydroxyethyl methacrylate) (pHEMA) [28] have been reported. Zwitterionic phosphorylcholine is integral to the outer red cell membrane surface, and can confer a degree of haemocompatibility [29]. Other biologically inspired molecules have also shown some effectiveness. Surface immobilised heparin [30] was used, and a NO adduct within a membrane released thrombus countering free NO [31]. Heparin had the facility to bind antithrombin and thereby to concentrate its anticoagulation effect at the surface, and the ubiquitous signalling molecule provided surface protection

through its platelet passivating action. As with any small molecule agent, including the mediators used for second generation enzyme biosensors, whenever a new component is contemplated, the risks of agent toxicity need also to be considered alongside the analytical benefits.

A further factor for any intravascular component is progressive thrombosis at points other than the sensing surface. The results could be flow blockage or disseminated thrombi to distant tissue locations. Materials for surface biocompatibility are often tested over only limited periods, and *in vitro*, so whatever promise is shown, may not transfer to *in vivo* deployment. One factor is vascular flow; platelets are highly sensitive environmentally, and become activated even by local turbulence, so surface deposits may occur under flow disruption regardless of a high materials surface haemocompatibility.

Future designs for ISEs, if used intravascularly, will need an integrative approach whereby not just the sensing surface, but also the flow compatibility of the entire construct will need optimsation. The starting point, though, is the appreciation of the scale of the *in vivo* biological challenge with blood. Testing with blood sub-components in focussed studies and under controlled conditions can only serve for initial screening. There is a limit to the practical value of such a reductionist strategy, and an ever-present risk of overoptimistic assumptions about a new material or surface delivering almost what was needed. Whole blood is a high alert, rapid response system that features multiple cooperative systems. It is able to harness a combination of cellular effectors like a tissue and is not dependent on diffusible, humoral signalling agents. It has been evolved to recognise, package and potentially degrade any foreign surface intrusion, both as part of fail-safe haemostasis and as a means of partitioning any 3D object within the circulation. Later parts of the blood response, in fact, begin to resemble those of tissue. From a first, transient, foreign surface encounter, it generates a coagulation cascade for thrombus formation accelerating through multiple enzymes and finally reaching an explosive rate in its mass generation of the final fibrin crosslink. It also has a complementary complement cascade that delivers surface coating protein (*viz* C3b) the promotes phagocytosis, amongst other effects, including promotion of inflammatory change. ISE *in vitro* to *in vivo* transition is challenging and warrants greater symmetry in blood *vs* sensor research if the early gains of sensor design are to be translated to common clinical use. It may be attractive to consider an intermediate solution with the use of an extracorporeal sensor as part of an external blood circuit. However, this also is not a easy solution; whilst greater control over blood flow dynamics, coagulation and calibration are achieved, complicated pump flow control and sterility protection are needed, together with a cumbersome fluidic supply, so usage would only be appropriate to a high dependency clinical care environment.

2.2 Oxygen electrodes

After the adaptation of O_2 polarography to *in vitro* blood use, using the Clark electrode, the measurement principle has changed little. Electrodes use gas selective membranes able to reject other solutes and ions to retain an electrolyte film for stable cathodic O_2 reduction whilst also excluding cell and colloid from the sample to eliminate working electrode fouling. The membrane may, for example, be a hydrophobic polypropylene or PTFE. Miniaturisation for intra-arterial use has been achieved [32], but is difficult given the need

for seamless attachment of inert hydrophobic membrane material to the body of a device. Equally, chemical modification for surface haemocompatibility is difficult; functionalisation needs harsh treatment. Also, any deposited coating may delaminate, and for any treatment a residual exposed hydrophobic surface will promote fouling through the extra tendency of a hydrophobic surface to denature adsorbed protein. Denaturation is more likey to trigger a host response than a non-denatured adherent protein. Surface fouling for an oxygen sensor is important here because response requires a continuous, stable flux of oxygen to the surface for a steady state response, in contrast to the ISE.

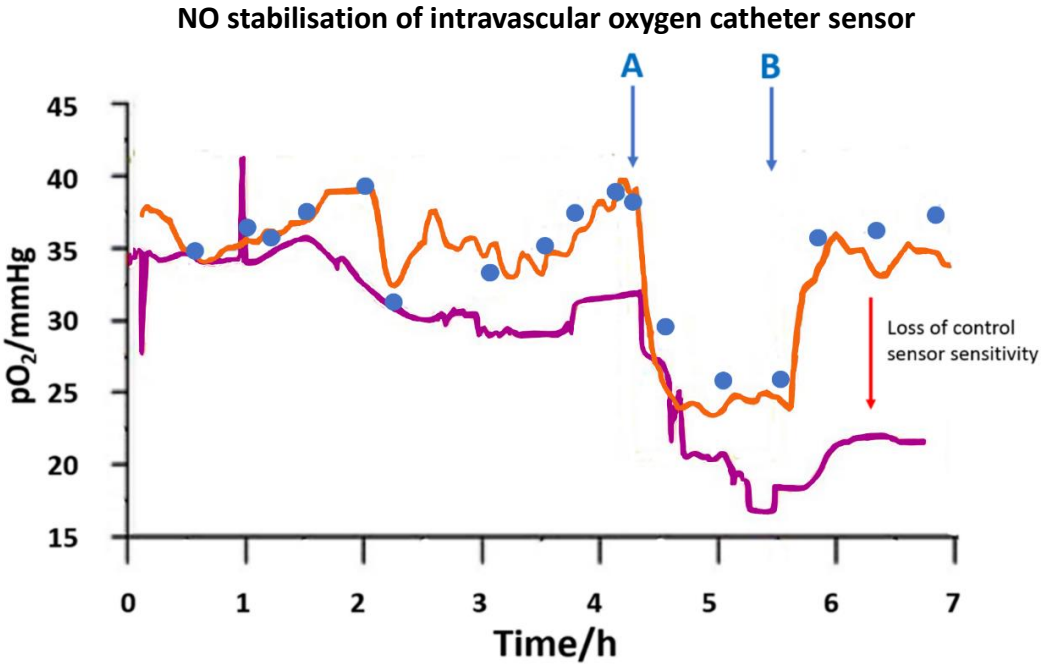

Fig 2 Double lumen intravascularly placed O_2 catheter with haemo-protective NO delivery used in rabbit jugular vein. (–) NO flow protected O_2 sensor, (–) control O_2 sensor. Blue filled circles are intermittently sampled venous blood pO_2 values assayed by in vitro analyser. A) 100% inspired O_2 was switched to 21%. B) Return to 100% inspired O_2 Adapted from [35].

Vascular catheters as a monitoring route were reported [33,34] in early studies, and a more structurally refined catheter model has been one that uses the catheter wall itself, notably silicone, as the gas membrane. NO release at such an electrode can offer a degree of suppression of surface coagulation. One embodiment used a double lumen silicone cannula where NO was electrochemically released from a second lumen nitrite reservoir [35]. Here, stabilised O_2 output was seen during acute monitoring of hypoxia (Fig 2). Surface coagulation, though not entirely eliminated, could still be sufficiently low for extended monitoring subject to a maintained NO release. At such a blood contacting device there is also the risk, in principle, that blood cells, especially nucleated cells, which are highly active metabolically, can act as an oxygen sink, so depressing measured O_2 values. Platelets are also active in this regard, though not mitochondria deficient RBCs. Ultimately, the blood interfacing problem may be amenable to resolution through synergistic use of locally delivered and surface retained anticoagulant agent, along with new catheter shape designs to sustain normal blood flow profiles. Alternatively, an extracorporeal, multiparameter system is available for

neonatal use (VIA LVM Blood Gas and Chemistry Monitoring System, VIA Medical) [36]; blood flow here alternates with heparinised calibrant enabling regular, automated, re-calibration.

3 Blood as a reactive sample matrix

3.1 Protein surface interactions

Blood-surface recognition utilises multiple, complex pathways which are as yet incompletely understood. Both plasma proteins and the formed elements of blood, other than RBCs, have a high tendency to adsorb to surfaces. Protein deposition commences within milliseconds, and is later amplified through complement and coagulation cascades that deliver a high mass surface coatings. This is the start of the thrombus, and though it may be structurally indeterminate, it advances through highly organised, controlled pathways. The speed and multi-factorial nature of the process makes experimental study difficult. Consideration of the idealised situation of a single protein as a monolayer provides a model to understand the initial events. Immediately after deposition and attachment of a protein to any surface, conformational remodelling is initiated due to non-covalent binding interactions and desolvation changes. Essentially, this is protein denaturation, which leads to chain unravelling and dimensional expansion. These surface attachments are driven by enthalpy lowering, but since attachment also leads to reduced entropy, to compensate, the free loops unravel to increase entropic freedom. The molecule has been considered to have a 'loopy' conformation and hence its surface footprint increases in area. The extent of the unravelling process depends on time, and so for a given mass, the area occupied increases (Fig 3) [37]. If in a biofouling study, the assessment period is a short one, then in an idealised situation, there will be a higher surface mass per unit area than if the time scale is a long one when the protein molecules have expanded and fewer are needed for full coverage, ie jamming. This makes for uncertainty in study comparison. In the limit, all molecules unravel and molecular adsorption density reaches a finite low level.

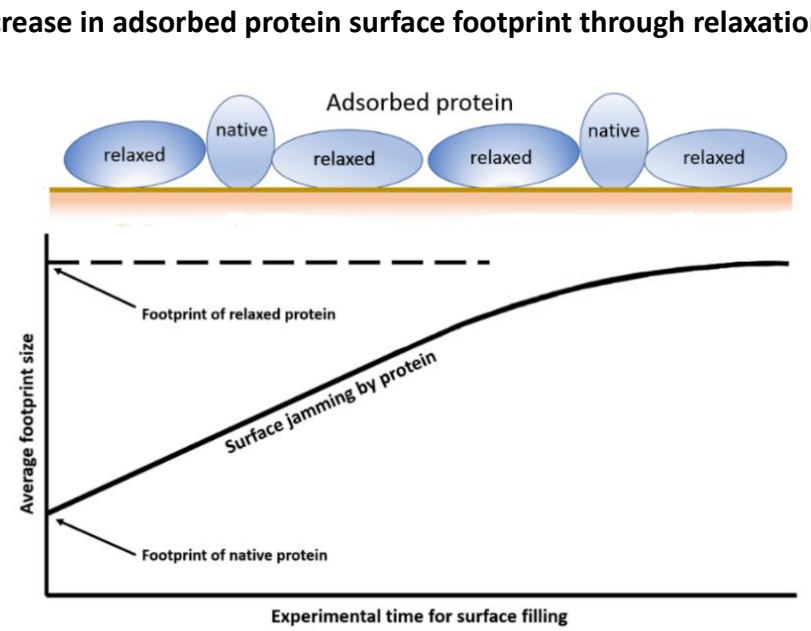

Fig 3 Schematic of progressive relaxation of adsorbed protein layer and increase in surface occupancy per molecule over time. Amount of protein needed for total confluent coverage (jamming) of surface is reduced as time of experimental observation increases. Adapted from [37].

The adsorptive behaviour of proteins from blood is orders of magnitude more complicated, but still initially driven by thermodynamics. The complexity is summarised by the Vroman effect [38]. In any multi-protein system, there is a competitive protein surface binding and exchange, not yet fully understood. Early protein adsorbates from certain high concentration proteins are displaced by slower arrivals with stronger surface affinity. Typically in this, albumin is replaced by fibrinogen. This shifting protein interface creates a changing contact surface, and is also the trigger for later biologically mediated cell and fibrin coagulum deposits. Some protein denaturation is inevitable at any surface, and this is a stimulus for blood activation through the presentation of new protein motifs (epitopes). A different type of protein accumulation is activated later *via* the complement cascade, and is designed to facilitate cellular phagocytosis: opsonisation.

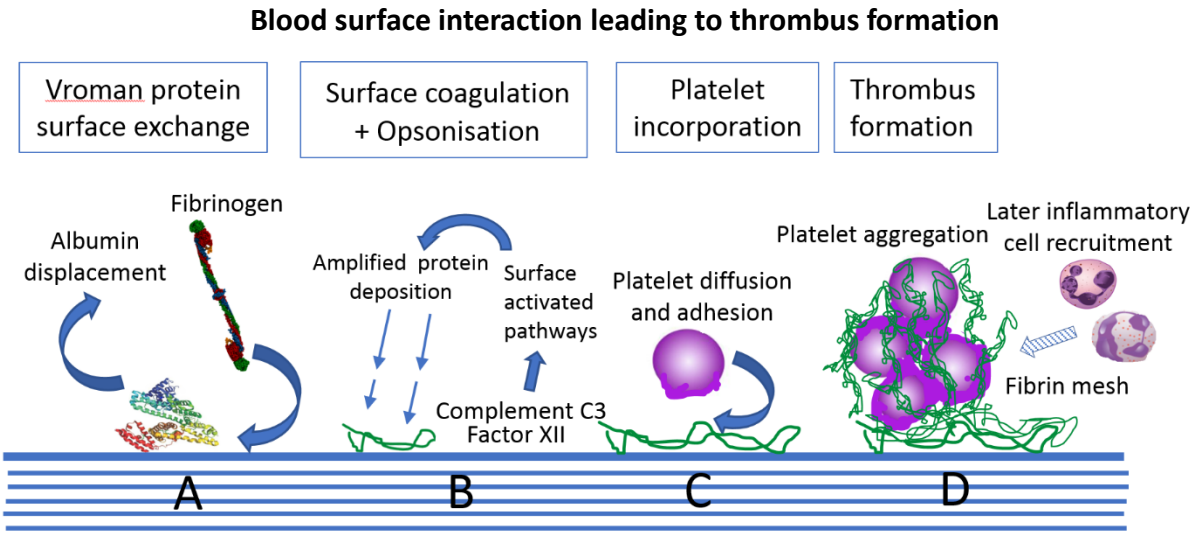

Fig 4 Schematic of surface coagulation sequence. A) Initial rapid protein deposition, in milliseconds, subject complex, competitive displacement/remodelling via the Vroman effect, eg fibrinogen displacement of albumin. B) Surface activated C3 and Factor XII trigger complement and coagulation cascades, leading to protein/C3b coating (opsonisation) and fibrin directed at the surface. C) Platelets contact with coated protein sets of adhesion response. D Platelet adhesion leads to activation and promotion of fibrin clot, later inflammatory cells incorporated.

Efforts to ultimately achieve zero protein adsorption seem unrealistic in respect of effectiveness against whole blood biology. An interesting alternative has been suggested by Brash [39]. This envisages that if deposition cannot be avoided, then surface directed selective protein deposition might be effective. Thus, a surface might be able to selectively invest itself with a functional property such as fibrinolysis (plasminogen adsorption) or anticoagulation (antithrombin adsorption). Reports on haemocompatible sensor surfaces indicate a continued quest for the single 'magic bullet' solution where none may exist. General rules, nevertheless, may be derived from such studies such as general rules for hydrophobic/hydrophilic balance for lower fouling and selected surface chemical properties that might link to complement activation [40]. The surface protein profile together with its later remodelled form [41] presents the real contact layer for subsequent cellular processes organised by blood, and from the start the original engineered or chemically modified surface ceases to have direct blood contact, and yet the response of blood to remains as log as it is in place.

3.2 Blood biological reactivity

Following the protein interaction stage, the intrinsic coagulation pathway is initiated by Factor XII surface binding. Complement C3, the core driver of the separate complement cascade, and causes independent protein coating and opsonisation. Complement C3 is triggered to fragment autocatalytically and produces C3b adsorbate for surface opsonisation; a surface that is now an attractant for inflammatory cells [42]. There is cooperativity between the coagulation and complement pathways, and this later leads to the incorporation of inflammatory cells into a developing surface thrombus (Fig 4) [43,44].

The platelet is the specialist element of blood that really drives the development of a surface thrombus and is one of its most prominent constituents. Its study is difficult because of its environmental reactivity, including to the very surfaces used to handle it, and its high sensitivity to flow induced stress. Its response to the developing coagulum is, moreover, quite specific, and involves receptor stimulation. This leads to dramatic change in a platelet, including degranulation, shape alteration to a discoid and multiple bridging/aggregation. This super-structure of platelets and entrapped fibrin then adds to the growing thrombus [45]. For sensors, though the focus has been on surface chemistry, surface physical profile may also be important. In one concept, a surface roughness of platelet dimensions (~2um) was considered to offer a better match for platelet surface contact and therefore thrombus formation than lower dimension roughness giving less platelet purchase [46]. Leukocytes in blood also become surface activated [47], are then recruited into the thrombus and promote coagulation through cytokine release.

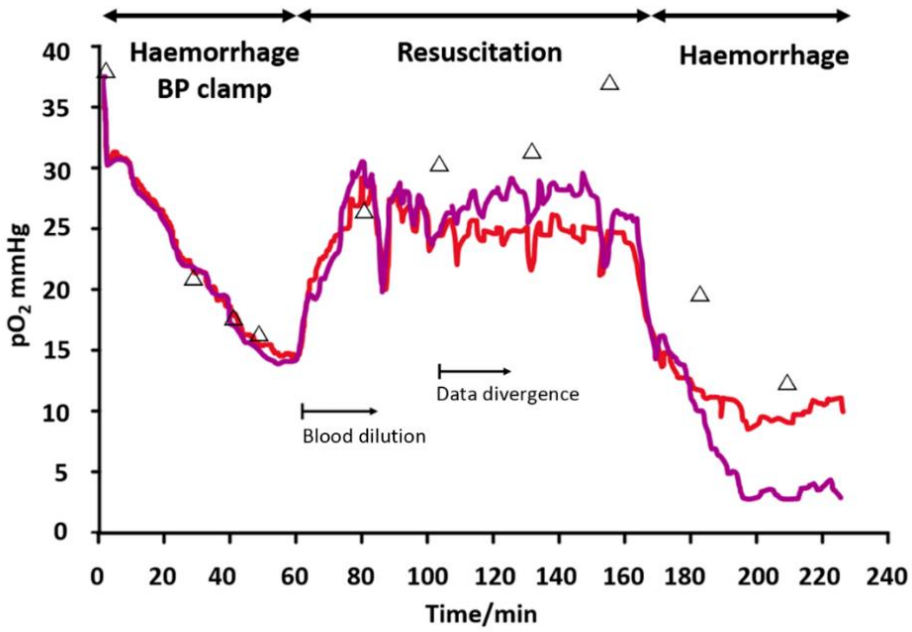

Fig 5 Tissue pO_2 changes monitored in single rat during haemorrhagic shock. Sensors at matched implantation sites in flank. Initial haemorrhage clamped at reduced BP (40mmHg); saline only resuscitation stabilises BP (60-70 mmHg); haemorrhage to exsanguination with extreme, terminal drop in BP. Resuscitation regimen would lead to cumulative blood dilution, progressively lowering oxygen carrying capacity to peripheral tissue. Adapted from [49].

4 Tissue oxygen electrodes

4.1 Compartmental difference

The Clark pO_2 sensor has also allowed continuous monitoring of subcutaneous tissue pO_2 [48]. Such a device has enabled tracking of peripheral tissue pO_2 during haemorrhagic shock [49], but there are indications that there are compartmental differences between blood and tissue. This is suggested for this study by an inter-sensor agreement that was greater than with venous blood (Fig 5). Measured tissue pO_2 was significantly lower than that of blood at the later part of the experiment, a possible outcome of subcutaneous circulation shut down. Blood pO_2 may reflect more general tissue values, eg of the more protected central organs, but further studies ae warranted. These changes cannot be unravelled from sensor drift, but that was the cause, then the polyurethane membranes used would be exceptionally lacking in biocompatibility, moreover, time was allowed for electrode stabilisation post-implantation; here, this took two hours. This is the norm, and considered an artefact with all sensors, without clear explanation given over mechanisms. These may be complex, but the possibly of a tissue functional response, eg microvascular change cannot be ruled out. Mechanical tissue damage and microhaemorrhage will certainly occur, but cannot be the full explanation. Calibration uncertainty in tissue lends uncertainty to actual tissue pO_2 values, which in any case will have local differences at the micron scale. Venous rather than arterial blood comparison was used for this study, though arterial pO_2 is the benchmark for clinical use. Arterial changes were only found at a very late stage haemorrhage, with venous blood, derived from tissue, more to reflect sensor changes.

The determinants of tissue pO_2 under non-haemorrhagic conditions was reported by Gough using a silicone membrane covered electrode [50,51]. Again, the same stabilisation delay and uncertainties about tissue O_2 were observed. They attributed variation in output at an array of tissue electrodes to local difference in vascular flow, and observed slow to rapid fluctuations of tissue pO_2, which they attributed to perfusion variation due to local vasomotor vascular control. The challenge with tissue is that of extracting valid physiological information in the face of an evolving tissue reaction, essentially a wound site. Surface biofouling raises a further uncertainty. A model for oxygen mass transport to the electrode was established [51] which indicated that sensor response was limited by local mass transport resistance, whilst more remote oxygen delivery was rapid being associated with blood flows. The high permeability membrane used here allowed resolution of such extra-sensor effects. Over 13 weeks these flux sensitive electrodes picked up a tissue reaction that led to decay in tissue O_2 permeability, to ~10% of that in water. Even the local fibrous capsule build-up to 5mm depth apparently did not completely abolish diffusive transport. Whist such a high permeability membrane can allow investigation of the tissue effects, practical monitoring requires a diffusion limiting membrane to negate external transport variables. Even here, over a long time period, it may prove difficult to achieve this negation if a substantive fibrous capsule forms. With tendon as a model dense collagen barrier, we found micro-solute diffusion to be just 1% of that in water [52].

4.2 Tissue micro-heterogeneity

Tissue oxygen delivery distribution at the microscopic level is a field in its own right, and numerous mapping studies of oxygen have been undertaken using microelectrodes [53,54]. Micro-heterogeneity is a result of cell uptake, vascular delivery and transport across the extracellular compartment. Cerebral tissue has been a particular focus because cortical blood

vessels are easily visualised, allowing combined analysis of vascular organisation and pO_2 distribution. In one study, a <5um diameter recess tip electrode with a collodion membrane was used to determine blood pO_2 along a sequence of arteriole, capillary and venule, along with perivascular oxygen distribution [55]. This showed not only the expected pO_2 reduction along the blod vessel sequence, but steep perivascular oxygen gradients extending ~60um into tissue to give pO_2 reduction of up to 80% intravascular. (Fig 6). Muscular arterioles of the CNS are unique in providing through wall tissue oxygenation, so there are gradients around these vessels too. Such work offers insights that may be useful for neurosurgery with a detailed picture of oxygen profiles in CNS tissue. Additionally, micro-delivery of vasodilator pharmacological agent to a single vessel showed that with relaxation of the arteriolar wall, through wall oxygen delivery increased.

CNS tissue axial pO_2 profiles around arteriole, capillary and venule

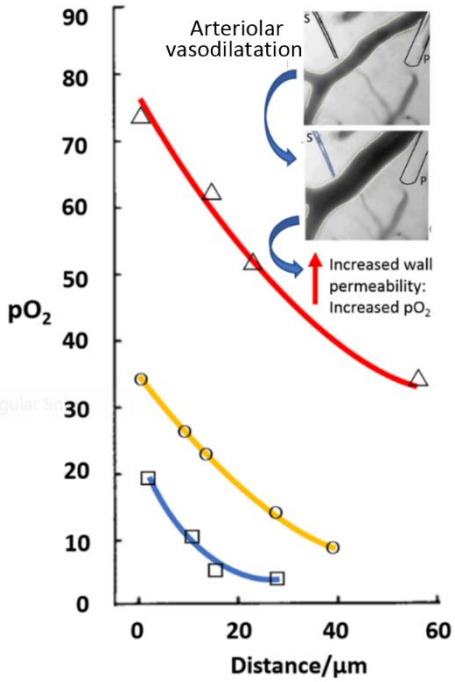

Fig 6 Rat cortical tissue pO_2 at varying radial distances from (—) arteriole, (—) capillary and (—) venule using 4µm tip oxygen sensor showing exponential reduction with distance from vessel axis. Inset shows microsensor (S) on 30µm diameter arteriole with subsequent vessel dilation after vasodilator delivery from nearby micropipette (P). Adapted from [55].

The question arises as to what macro-electrodes can tell us. They offer a 'field of view' extending hundreds of microns and therefore a sample aggregate of different tissue oxygen micro-sites, blurring fractal complexities. The uncertainty is what the exact size of this operating might be. The issue is typically bypassed by setting an empirical *in vivo* calibration against blood pO_2 from the start. The outcome is still meaningful trend monitoring of pO_2, as for clinical purposes during compromised tissue oxygen delivery [56-58] and for assessing cardiac oxygenation dynamics during ischaemia/reperfusion [59]. A commercial electrode is available for specialist CNS use (Licox, Integra Life Sciences Corporation) [60], but there is, as yet, no general tissue electrochemical sensor. This commercial system samples through an extended $18mm^2$ area polyethylene tube to capture gross regions of brain. Again,

uncertainties emanate from the interrelationship between microcirculation organisation, flow, vascular distance and mass transport, all balanced against cell metabolic uptake [61].

Beyond the validation of sensor stability through pre- and-post use calibration, there is no simple means of establishing the true pO_2 experienced by a device [60]. This is where need for disease correlates and diagnostic benefit diverge from the rigour of measurement science. Indication of the degree of uncertainty is shown by reported differences in monitoring output when the principle of measurements is changed, eg from electrochemical to optical. However, trend monitoring is of distinct value, clinically; in the case of head injury, for example, measured hypoxia correlates with outcome.

The CNS is a relatively implant tolerant tissue, a contrast to subcutaneous tissue which demonstrates a florid, cellular inflammation. However, at the opposite extreme is when inflamed tissue is deliberately probed. The pO_2 of inflamed synovial tissue in rheumatoid and psoriatic arthritis patients was measured with the Licox probe. Variable degrees of hypoxia were seen, and the degree appeared to correlate with disease severity. There was even the suggestion that hypoxia was a driver of the inflammation, as manifest by the degree of oxidative damage and the degree of hypoxia, and through the vascular damage and prevalence of T cells and macrophages [62]. Consistent with this possibility was the improvement in oxygenation seen following anti-inflammatory therapy; pO_2 doubled from ~20 mmHg in responders. A pO_2 correlation was also demonstrated for T cells, but not for B cell infiltration, indicating a disease causal link with the former. An inflammatory milieu anything like to this degree at an implant site would radically change measured pO_2, no longer reflecting systemic levels. Despite the oxygenation correlations seen here with histology, there is also the likelihood that high respiring cells simply caused a low tissue oxygen and measured levels were simply a functional reflection of the cell population.

pO_2 distribution microheterogeneity in normal *vs* cervical cancer tissue

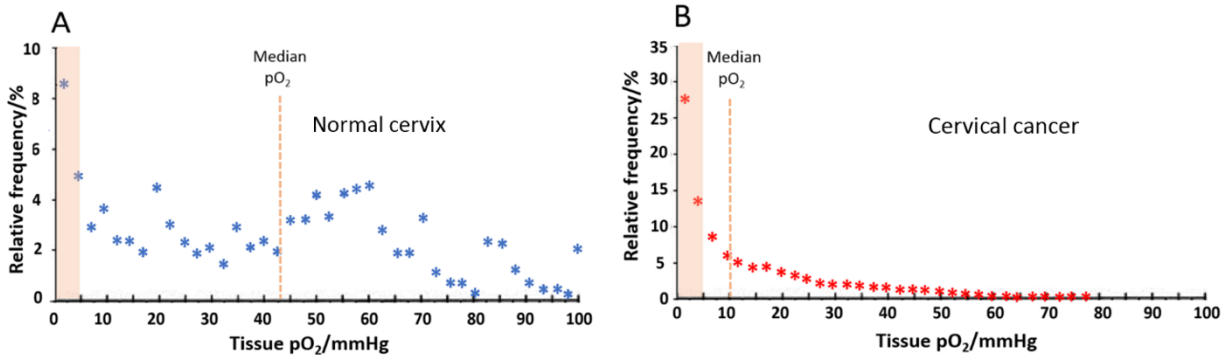

Fig 7 Percentage frequencies of pO_2 measured across A) Normal uterine cervix (7 patients, 432 samples), (B) Cervical cancer (150 patients, 13596 samples). Shaded areas highlight percentage prevalence of extreme tissue hypoxia of <5mmHg. Each set of data represents combined data points from multiple patient samples. Adapted from [65].

4.3 Cancer tissue

Solid cancers grow rapidly and can outgrow their vascular supply, already compromised through disordered, dysfunctional blood vessels. Zones of hypoxia arise which has clinical relevance, because hypoxia confers tumour radio-resistance. A range of analytical techniques

have been employed to study cancer blood supply and oxygenation and the field has been reviewed [63]. O_2 microelectrodes, in particular, have made it possible to unravel the oxygenation architecture of cancer tissue. A commercial recess tip oxygen electrode (Eppendorf, Hamburg, Germany) housing a 17um gold cathode and a PTFE barrier layer enabled sequential tip positioning through tumour tissue at aligned 0.6mm distances [64].

This generated histography data (histography), when combined for tumours from many patients in the case of cervical tissue, showed up a stark oxygen distribution difference for normal *vs* cancer tissue. For cervical cancer, for example, median pO_2 was a mere 10mmHg, compared with normal cervix at 43mmHg, with also a huge preponderance (>60%) of exceptionally hypoxic microenvironments (Fig 7) [65]. It is notable that even in normal tissue, sites of near zero oxygen levels exist. A hypoxia link to cancer prognosis, however, appeared to be absent, but there is value to such study of the oxygen state of cancers.

5 Glucose electrodes

5.1 Monitoring needs

Glucose remains the most important target for continuous monitoring. Whilst elegant studies exist on the mechanisms and optimisation of enzyme-electrode electron exchange, these alone cannot alone translate into operational benefit. Commercial development has helped advance this latter operational aspect, initiated with single use strip design [66]. Since the early days of glucose sensor development, diabetes has escalated in prevalence, and so glucose sensing has risen up the health priority list, along with this, interest in continuous monitoring. Diabetes now poses a massive healthcare burden currently affecting 9% of the global adult population. In this group are the 5-10% Type I diabetics [67] needing insulin who warrant close monitoring to manage histography their therapy. They are also liable to marked glucose fluctuations, eg during concurrent illness when in insulin sensitivity changes. The brittle diabetes sub-population within this group, though small, has highly unpredictable insulin needs and difficult to manage glucose levels; these patients are prone to dangerous hypo-/hyperglycaemia [68]. So continuous glucose offers specific benefits to the Type I diabetic, with a reduction through improved glucose control of long term vascular and other complications.

Sensors when used as single use devices allow for greater design flexibility permitting, for example, genetically modified enzyme, leachable/soluble mediator and a modified electrode surface. The major consideration here is high shelf life stability and calibration-free measurement. Beyond that, survival in blood need only be for seconds. An *in vivo* sensor, by contrast, may be allowed high calibration variation pre-use, but beyond that, stability in use has to be sustained over long periods, and the electrode surface, enzyme component and any incorporated reagent components need to be guaranteed to be safe, so flexibility over design chemistry is limited. Also, intravascular sensors risk disseminated damage, so despite the uncertainty tissue is now the target site for continuous glucose monitoring (CGM).

5.2 Clinical realities

Measurement based on O_2/H_2O_2 tracking of the glucose oxidase reaction is the basis for most CGMs, avoiding additional chemical components and mediators. The exception has been the osmium based electron shuttle developed by Heller [69], now used for clinical CGM (FreeStyle Libre, Abbott, Almada, US). The sensor is accepted for 14 days use, and ultra low (~2%) drift

independent of calibration has been reported [70], considered to be on the basis of a high biocompatibility covering membrane. Even during normal dynamic glucose changes there was stated here to be concordance with blood glucose values. This system, however, enters a new type of unknown into the measurement; devices are manufacturer calibrated to generate automatic blood equivalence [71]. This brackets a series of known variables including blood *vs* tissue glucose relation within and between patients, glucose dynamic change modulation of this relationship and also any implant site dependence. Studies have shown that all are variables affecting response, and should be considered as factors that need to be allowed for on an individual basis. Nevertheless, the clinical value of this approach has been recognised through improved monitoring benefit to the patient.

Generally, in the literature the true measured glucose value in tissue is bypassed, as with oxygen, and the starting point for data recording is after calibrated against blood *in vivo*. This is really a form of data ratioing across compartments rather than a true calibration, and provides no actual information about the tissue state. Modelling of plasma-tissue exchanges by contrast recognise delayed and variable exchange kinetics and the need to factor in genuine lag times to underpin data correction [72,73].

Added to the physiological uncertainties, there are changes due to the reactivity of tissue at the implant site. As yet, no material has been able to claim the stealth performance needed to eliminate the disruptive tissue reaction, despite the many sensor design iterations [74-76]. Performance decay is also maximum in the hours following implantation; the so called run-in period of hours to days which warrants separate consideration.

5.2 Membranes and coatings

The coatings and coverings for glucose sensors have mostly used existing materials. The key requirement is low surface fouling and stable glucose and oxygen permeability. Shichiri et al [77] were the first to demonstrate such packaging in their use of polyurethane in an implanted device. We and others have similarly utilised polyurethane [78,79]. Such repurposing of a medical polymer helps reduce the unknown risk of a new material and with appropriate porosity and diffusion control enables a response that is sufficiently independent of sample physical properties or oxygen background for the clinical glucose range. However, commercial CGM manufacturers have been able to develop and incorporate new materials, reviewed by McGarraugh [80]. The Guardian Minimed (Medtronic) employs a block copolymer polyurethane with a glucose permeable hydrophilic diol phase for glucose, balanced against a silicone that would presumably be O_2 only permeable; the DexCom (DexCom Inc) uses a hydrophobic/hydrophilic polyurethane mixture for balancing diffusive transport with presumably a similar differential permeability intended. Part of the design goal for materials here will be the minimisation of oxygen diffusion limitation for the enzyme reaction. In the absence of mediator transmembrane transport selectivity provides an important means of achieving this. The design challenge is the micromolar levels of oxygen concentration in tissue. The FreeStyle (Abbott) departs from the polyurethane platform and uses a functionalised vinyl pyridine-styrene copolymer, but here the mediator based device is O_2 independent. A claim made for the latter was of the absence of tissue encapsulation even at one year implantation, [80], though the muscle location might have been a factor.

Membrane innovations have also been reported for CGMs on an experimental basis. Moussey has advanced a range of compositions that have included a hydroxypropyl methacrylate hydrogel coating on polyurethane giving reduced inflammation and fibrosis [81], humic acid films that provoked less tissue reaction [82] and a structurally robust epoxy-polyurethane which though leading to a fibrous capsule also stimulated vascularisation [83]. In one study, a porous polyvinyl alcohol scaffold was used as a covering matrix on the sensor, and this took up collagen from the tissue surround, along with new blood vessels. However, the collagen barrier effect countered the supply benefit of increased vascularisation [84]. Nafion, a perfluorosulphonic ionomer has been extensively studied, and as a tissue contacting sensor surface, it has generated a reduced tissue reaction with only a thin fibrous capsule at three months [85]. A comparative study of negatively charged membranes as part of a sol gel layer utilised Nafion, dextran sulphate and polystyrene sulphonate [86]. Results were similar, with thin collagen capsules seen at 12 weeks foreach material. The lack of a difference is a reminder that chemical refinement does not necessarily change outcome. Neutral polyethylene glycol (PEG) has well recognised antifouling properties and as a tissue contact surface provoked less tissue reactivity with reduced cellularity and tissue adherence [87].

Phosphoryl choline as a cell membrane zwitterion has again been used to reduce protein and cell deposition in blood at an intravascular glucose sensor located in the carotid artery of rats [88]. This was an acute study, and long-term outcome would need to be investigated.

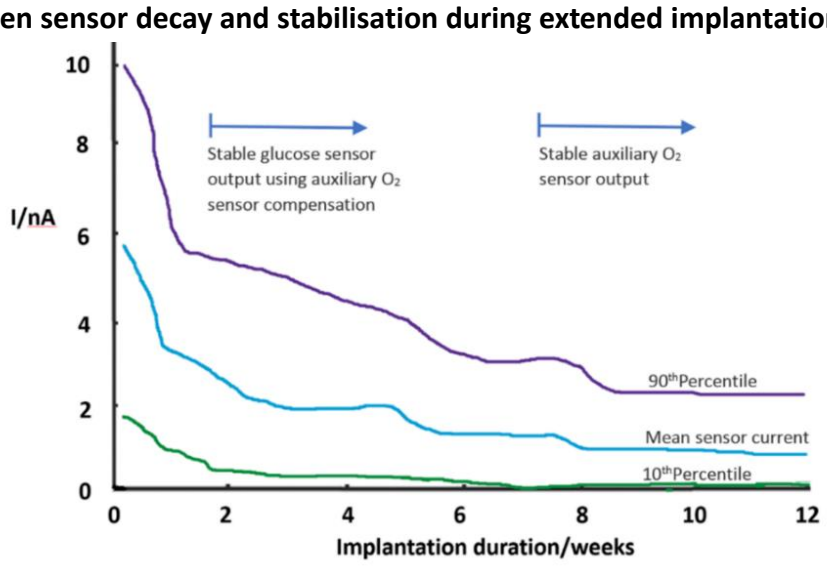

Fig 8 Auxiliary oxygen sensor current decay in subcutaneously implanted glucose sensors in pigs. Data represent 1 week moving averages of daily mean sampled currents and the spread of data for 60 electrodes. Adapted from [91].

Application has been transferred to tissue. Following initial combinatorial screening, a PEG crosslinked phosphoryl choline polymer was used over a commercial CGM sensor in mice and primate studies [89]. Inflammation mitigation by the phosphoryl choline reduced the need for repetitive *in vivo* calibration. Blood to tissue glucose mismatch was reduced, though there was still late fibrous capsule development. Phosphoryl choline translation from blood to tissue would be a valuable future direction. Here and for other studies, the possibility cannot be excluded of changed surface mechanics, especially with a gel. Tissue is reactive to surface

mechanical cues. Whilst not necessarily due to mechanical change, in one study, soft electrospun gelatin coatings on polyurethane fibres over sensors reduced fibrous encapsulation, compared with non-coated fibre [90].

As an alternative to the registration of H_2O_2 product, Gough advanced the use of cathodic O_2 measurement. In one study, a surgically fully implanted sensor was operated for a year [91]. A dual sensor arrangement was necessitated here, with the second, non-enzymic, O_2 sensor compensating for background oxygen variation. Glucose oxidase was used in a crosslinked gel, and whilst there was a tissue reaction and a steep response decline with the secondary oxygen sensor (Fig 8), the dual O_2 approach enabled glucose monitoring from two weeks. The wide statistical spread seen in responses for different oxygen sensor implants indicated the variability of the local tissue response. Whilst a protracted stabilisation delay is an option, it would seem so only if long term implantation is contemplated, and that demands a high level of confidence in sensor that would need surgical implantation. A subsequent six month human study with these sensors demonstrated stable oxygen compensated glucose tracking [92]. Collagen capsule imposed response delay was of the order of 10 min, so workable for clinical purposes.

5.3 Bioactive molecule release for biocompatibility

Drug loaded membranes have been reviewed by Ahyeon et al [93]. Dexamethasone, a high potency steroid can suppress inflammation and late fibrosis; VEGF (vascular endothelial growth factor) can promote vascularisation to augment glucose delivery. Biological complexities may be seen, however. Vallejo-Heligon et al [94] found dexamethasone loaded polyurethane to both suppress inflammation and to promote neovascularisation, extending the sensor operating period; its combination with VEGF, however, led to depression of VEGF induced neovascularisation [95]. Despite a high early effectiveness of such loaded membranes, reservoir depletion is a potential drawback, especially with thin, low capacity membranes. One study instead delivered VEGF from a cannula using an osmotic pump [96]. Neovascularisation was demonstrated which was evident to at least 40 days at a tissue distance of 1.3 cm. Composite material designs might also extend function life time time. Dexamethasone when loaded onto poly (lactic-co-glycolic acid) (PLGA) microspheres of two molecular weights provided early and late release with bioactivity retained for 6 months [97], determined by the differential degradation rates of the two polymers. With microspheres embedded in porous polyvinyl alcohol (PVA) surface fouling was offset by porosity recovery as the microspheres degraded [98].

Bioactive NO releasing membranes have also been developed [99] with the aim of suppressing pro-inflammatory cytokine release and thereby inflammatory cell recruitment. Stabilised sensor operation depends again on a maintained NO reserve. Interestingly, here, the sensor run-in period was short. A NO effect might offer a clue to this with perhaps a link to signalling. NO is electroactive at anodic voltage, but if its interference is constant then monitoring would be possible. In regard to this, the authors postulated that a variable generation of NO by inflammatory tissue might be a contribute to response instability.

The widening repertoire of clinical therapeutic agents, including biologics, should provide a new generation of anti-inflammatory agents. Masitinib a small molecule agent used to treat mast cell tumours is one example. Mast cells are central to the tissue response; they are

immediately available, undergo ready degranulation and variously release proinflammatory cytokines, serotonin and histamine, and so accelerate inflammation. Masitinib works by blocking mast cell receptors, thereby suppressing intracellular kinase signalling and stabilising the cell membrane. When used as a release agent in a sensor [100], however, degree of limited protective effect was demonstrated, but not as much as might have been expected from drug potency.

5.4 Tissue reactivity to implants

For bulk dependent implant devices, the tissue reaction is of a lower order concern, but for surface response dependent sensors, even a minor reaction can have profound effects. Looked at in the opposite sense, tissue is actually the more sophisticated sensor. Thus, it has mobile surveillance through its constituent cells, a strong capacity for recognising even the smallest of foreign body intrusions as 'non-self' and an ability to resolve shape; any 3D object, however it is packaged, or disguised is readily recognised. From this recognition starting point, a cascade is established, embodied in the Foreign Body Reaction. This is designed to degrade the intrusion, and failing that, to package and isolate it behind a fibrous capsular wall. It is too fundamental a part of the armoury of an organism, linked to its very survival, to be readily countered. While the outcome for the technology is adverse, for the biology it is an unmitigated success. The current state of the art is that whilst we have dissected the complex response pathways, it is our understanding of them that lags behind [101,102].

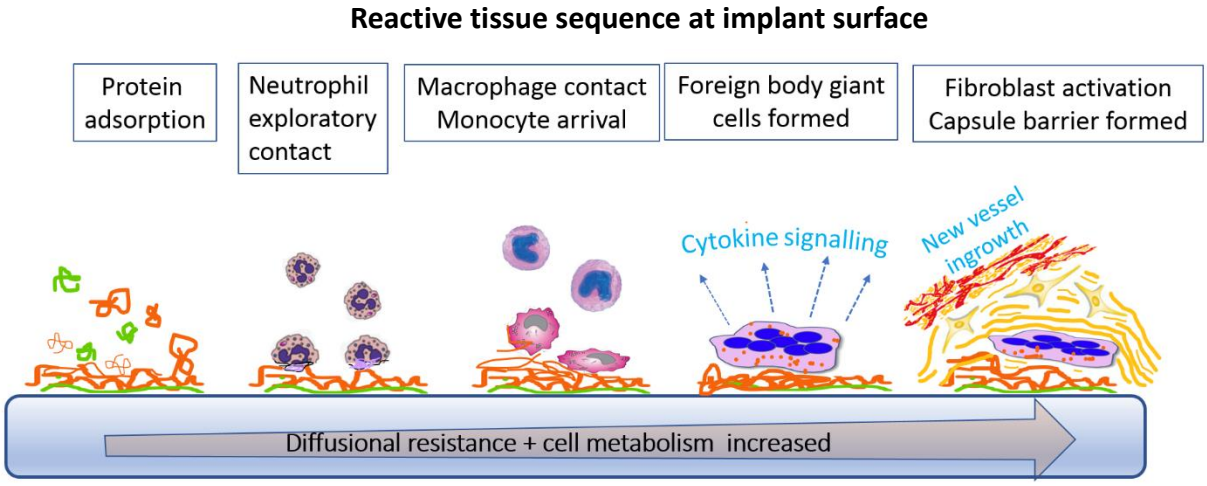

Fig 9 Schematic of tissue foreign body response in sequence: 1) Rapid protein deposition masks sensor surface; deposited layer increases. 2) Tissue neutrophils sense the surface and send chemotactic signals, mast cells promote inflammatory background. 2) Macrophages accumulate with population reinforcement by blood monocytes. 3) Failure to degrade surface stimulates more powerful multinucleated giant cell formation from macrophages, with enhanced signalling. 5) End stage of more quiescent collagen formation and cumulative barrier formation by fibroblasts with parallel neovasularisation.

On implantation in tissue, as in blood, rapid protein deposition takes place and the device is already packaged by a layer that though conditioned by the original surface, itself goes on to condition further responses (Fig 9). Early protein reorganisation, layer accumulation and denaturation characterise this growing protein layer. The tissue cellular tissue response is subsequently effected by specific receptor binding to these adsorbed proteins. The first responders are exploratory polymorphonuclear leucocytes (neutrophils), and with mast cells

they initiate diffuse chemotactic signalling to attract other phagocytic cells (macrophages). These amplify the directed signalling and recruit even more macrophages. Factors released include PDGF (platelet derived growth factor), TNF-α (tumor necrosis factor alpha) and IL-6 (interleukin-6). Within this mix are added monocytes from blood, together with proinflammatory mediators, replenishing the macrophage pool. If phagocytosis against the device fails, macrophages on the surface fuse to make more effective multinucleated foreign body giant cells through a trigger that is unknown [103]. There is also an outpouring of degradative agents with no other purpose than to solubilise the intrusion, which includes acidic cell contents, oxygen free radicals and hydrolytic enzymes. Ultimately, degradation may be a highly desirable biomaterials outcome if the agent is a surgical suture, but if it is a sensor membrane it becomes a clear problem. Preferential degradation of the soft segments of a polyurethane used for glucose sensors illustrates this [104].

If the implant stimulus persists over days or weeks, a more cellularly heterogenous inflammatory cell architecture is built up with added lymphocytes and plasma cells. Healing then ensues if there is no outright toxicity. This progresses behind a cell layer adjacent to the device, and variously hosts a dense network of inwardly directed blood vessels, fibroblasts and macrophages: granulation tissue. This is also the remodelling phase of the response, and a precursor to final collagen capsule deposition by fibroblasts. This sequence of events around a non-toxic implant is pre-programmed constant, refractory to management with only its quantitative aspects varying across different materials [105] or through suppression regimens.

5.5 Tissue reaction implications for glucose sensors.

During implantation, some microhaemorrhage is inevitable. The locally released RBCs can then become a sink for glucose, though not for oxygen. After early RBC removal and the entry of more actively metabolising nucleated cells, glucose and oxygen access to the sensor can both become reduced. Direct injection of macrophages to a sensor implant site reproduced this effect [106], but interestingly not lymphocyte injection despite the metabolic activity of these cells. Within days, a rapid population change occurs with an order of magnitude expansion of neutrophil number followed by a decay and a parallel increase in lymphocyte number. No difference in this tissue response was seen in one study, whether or not the sensor was operational and releasing H_2O_2 into tissue [107]. What is a constant with all devices is that final fibrous capsule formation is inevitable and becomes the arbiter of what is then seen by the sensor. Studies of beyond a week confirm such capsular development and its effect on glucose exchange, in one example leading to a 24min delay following intravenous glucose [108]. There is a match here also with the tracked arrival time of injected fluorescent glucose analogue. Real physiological exchange between blood and tissue is considered quite rapid, requiring <5min for completion, so implanted sensors clearly create an artefactual delay.

The collagen capsule, far from being a simple, static accumulation of collagen fibres, is an evolving structure with its own vascular network and an internal palisade of cells apposed to the sensor. Novak et al [109] took this structural duality into account in their modelling of glucose transport. They concluded that lag time was determined by capsular thickness whilst sensitivity was a function of capsular porosity and local vascularity. Additional effects of macrophages and adipocytes metabolism were small here. By contrast, glucose losses

due to local cell metabolism were evident in a cell loaded fibrin gel, further accentuated by exposure to pro-inflammatory agent [110], highlighting again the cellular influence of an inflammatory matrix.

Glucose needle sensor combination with tissue microfluidic delivery

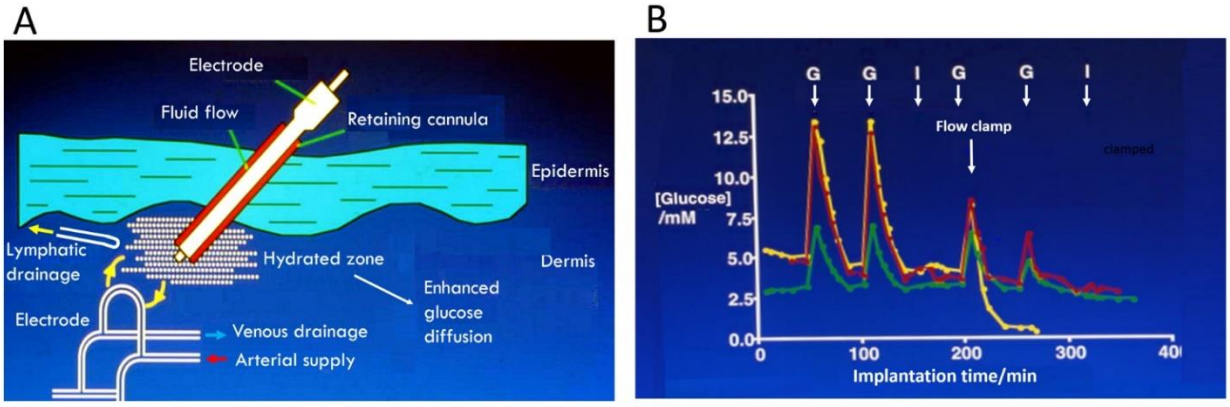

Fig 10 A) Schematic of subcutaneously implanted glucose needle electrode within open ended cannula for delivering fluid around the implanted sensor to create a limited hydrated zone. B) Subcutaneous glucose monitoring in rat (●) venous blood glucose, tissue glucose at 60µL/h microflow (●) and at a constrained flow of 10µL/h (●) showing underestimated glucose and total loss of response with clamped flow. Bolus tail vein administration of glucose (G) and insulin (I). Adapted from [79].

Our own studies on implant sensor stability have led us to use a materials independent strategy. Whilst inflammation comprises a hypercellular environment with distinct histological characteristics, it is also a zone of high, histologically silent, fluid influx due to permeabilised capillaries delivering protein rich fluid that is passaged to the lymphatics, and part returned to the microcirculation. A balance of hydrostatic and osmotic transcapillary pressures driving the fluid flow as enunciated in the Starling mechanism [111]. The device here was designed simply to deliver extra protein-free fluid to the implant site. It utilised an electrode-cannula coaxial arrangement for low volume fluid delivery to the sensor tip (Fig 10 A). Subcutaneous tissue has a negative hydrostatic pressure, and the arrangement enabled pumpless tissue driven low flow. The resulting locally reduced protein load reduced sensor fouling and stabilised response. Response lag time with respect to blood was eliminated and measured tissue levels matched blood without *in vivo* correction.

So far from local glucose being diluted, glucose access was likely to have been enhanced through a well hydrated, open structure, interstitial tissue space (Fig 10 B) [79]. So the conclusion of the approach is that a fluidized zone of blood equivalence is obtained. As both a practical and model system, fluid management of the interstitium could provide an alternate means of manipulating the implant environment.

6 Lactate

As the end stage metabolite of anaerobic respiration, lactate offers a quantitative measure of hypoxic and shock states where the peripheral tissue O_2 supply is compromised. As such it has formed a core means of tracking the severity of such states and their response to treatment. It is inevitably subject to rapid change, but despite this, clinical continuous monitoring is not available. Some experimental work on lactate oxidase sensor based *in vivo*

monitoring in brain has been conducted. Here, oxygen co-substrate limitation at the enzyme could potentially lead to underestimated lactate levels, so an oxygen discriminating membrane has been one option [112]. Alternatively, stoichiometric regeneration of oxygen from H_2O_2 product has been tried using incorporated CeO_2 catalytic particles, and improved lactate response during brain monitoring in hypoxic rats [113]. As with glucose, without an independent reference measurement in tissue, a true tissue lactate level is difficult confirm. Direct validation is possible in the case of an intravascular sensor, and anti-thrombotic NO release from such a sensor for added device stabilisation has been reported [114]. Intravascular electrodes responded rapidly to both lactate administration and hypoxic challenge. With subcutaneous deployment, however, a substantially blunted and delayed response was seen. Moreover, in this pig model, upper *vs* lower body implantation altered responses. We have also found a blood-tissue discrepancy in shock and subcutaneous site dependent output [115].

The scale of the mismatch well exceeds that seen with glucose and suggests that at least under shock conditions, there may be an added barrier to lactate release from the circulation. This should not occur at the capillaries, which are not selective for micro-solute, but possibly in the interstitial tissue space which as a polyelectrolyte may create an ionomeric barrier to the lactate anion. In diffusion through cartilage, we found the diffusion coefficient for ascorbate anion to be a small percentage of that for similar size neutral molecules [52]. The complexities of blood tissue compartmentalisation are shown in a study of muscle interstitial tissue [116]. Here, microdialysis sampling demonstrated tissue lactate at rest to be double that of plasma water at rest, and converged with plasma during exercise elevation, whilst glucose at rest was about half that of plasma, but again converged during exercise. The results indicated that muscle was able to control its extracellular environment. Subcutaneous connective tissue will not have this capability. Our exercise study with tissue microdialysis did not make a comparison, but showed a blunted lactate response even delayed to the post-exercise period during which tissue glucose appeared to fall [117]. It is clear, therefore, that study of different interstitial locations and comparisons between techniques for lactate are needed to help understand intercompartmental exchanges, for if we do not understand these, our understanding of events in simply the blood compartment will be limited.

6 Conclusions

In vivo sensors purposes are a special sub-set of electrochemical sensors, and constitute a distinct practical offering compared to fundamental electrochemistry studies on, say, cell signalling and CNS neurotransmitters. Unlike many sensor types, including industrial, they operate not merely in a hostile environment, but one that is active, reactive and protean in its nature. This rather counters the idea of biology as a benign, tolerant matrix presenting mild solution conditions. Recruitment of high surface activity and destructive cells in high numbers renders the implant site far from representing the normal physiological state locally and provides evidence of a contrived effort sensor disruption. Our quest for data immediacy on some variables in the acutely ill patient, however, requires just such sensors.

There also remains a need for repertoire expansion into a broader palette of intermediary metabolites, as these interact dynamically, and will give added clinical information. Now we better understand compartmental differences there is an even stronger case for developing the tools for examining these separate entities at different locations.

Electrochemical sensors have been the mainstay of such endeavours, and this review has highlighted the insights they have given us. This reinforces the need to resolve the generic problem of biocompatibility. It has been an inappropriate quest in many ways to search for the single material or surface the absolves us of this problem – the result has simply been more model systems. The quest needs to be far more rooted in study of the reactive biology. If nothing else, we have learned that this reactivity is not surface restricted, has a signalling hinterland remote from the surface.

By addressing the right issues, electrochemical sensors will be able to expand service from physiology to precision medicine. Also, future development of closed loop feedback control and autonomous therapeutic management will become feasible. Much of the capability is in place, including sensing chemistry, it is the biological control of the implant site that remains to be resolved. A far better, multi-parameter, understanding of the individual's dynamic bio-signature might emerge, and this would be entirely in step with the needs of individualised therapy, currently limited genomic profiling work.

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