O₂ microsensors for minimally invasive tissue monitoring

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Tissue oxygenation is a key factor ensuring normal tissue functions and viability. Continuous real-time monitoring of the partial pressure of oxygen, pO₂, in tissues gives insight into the dynamic fluctuations of O₂ supplies to tissues by blood circulation. Small oxygen sensors enable investigations of the spatial variation of pO₂ in tissues at different locations in relation to local microvessels. In this paper, pO₂ measurement using microelectrodes and biocompatible sensors is discussed and recent progress of their application in human skin is reviewed. Emphasis is given to working principles of a number of existing oxygen sensors and their potential application in vivo and in tissue engineering. Results on spatial and temporal variations of the pO₂ in human skin introduced by localized ischaemia-reperfusion are presented when the surface of the skin is covered by an oxygen-free paraffin oil layer and the range of the tissue pO₂ is deduced to be between 0 and 60 mmHg. In the study, pO₂ increases from 8.0 ± 3.2 mmHg (n = 6) at the surface of the skin to 35.2 ± 8.0 mmHg (n = 9) at a depth just above the sub-papillary plexus. Temporal decay in pO₂ following tissue compression and rise in pO₂ following pressure release can be described using mono-exponential functions. The time constant for the exponential decay, τ = 8.44 ± 1.53 s (n = 7) is consistently greater than that for the exponential rise, τ ′ = 4.75 ± 0.82 s (n = 6). The difference in pO₂ change with the time following tissue compression and pressure release reveals different dynamic mechanisms involved in the two transient phases. The elevated steady state pO₂ following reperfusion, which is approximately 20% higher than the pre-occlusion value, indicates localized reactive hyperaemia. Possible applications of O₂ microsensors in diseases, e.g. tumours, pressure ulcers, are also discussed.

Keywords: microelectrode; biocompatible sensors; tissue oxygen partial pressure

1. BACKGROUND

The capability to monitor O₂ on a continuous real-time basis gives the opportunity to both assess dynamic fluctuations, and to make a predictive assessment of O₂ trends in any particular localized environment. Electrochemical and optical (fibre-optic) sensors provide a near-ideal means of localized tissue monitoring other than totally non-invasive methods such as the near-infrared monitoring of the inter-converting Hb/HbO₂ chromophore pair pioneered by Jöbsis (1977). In both electrochemical and optical sensors, a direct interfacial reaction takes place, which can be conveniently amplified and translated into a continuous electrical output using systems suitable for near-patient use. A key capability is that of virtually reagentless measurement in an optically opaque tissue matrix—ideal for subcutaneous measurement. With the advent of miniaturization techniques, including those adapted from the microelectronics industry, ethically acceptable, clinically invasive monitoring becomes feasible.

Application criteria include sensitivity, selectivity, stability, biocompatibility, reliability and overall safety. Undoubtedly, an important route to overcoming many of these problems was to retain sensors behind permeable membranes and appropriate encapsulants (McDonnell & Vadgama 1989). Interfacing with appropriate polymer membrane coverings has permitted the operation of many sensors independently of solution variables presented by the in vivo milieu.

2. ELECTROCHEMICAL O₂ MONITORING

The polarographic principle of electrochemical O₂ reduction at a noble metal polarized at approximately −0.65 V versus Ag/AgCl has often been used to produce working oxygen electrodes. However, in the Clark electrode design (Clark 1956), O₂ permeable membrane separates an entire (two electrode) electrochemical cell from the unmodified, undiluted biological matrix. This methodology has been the mainstay of implantable O₂ sensors. The membrane, typically of PTFE, has a crucial role in restricting O₂ permeability sufficiently to limit evolved diffusion gradients to within the physical

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in relation to the microcirculation. The additional advantage of using a microelectrode is the limit to the competition for $O_2$ consumption with cells in the immediate environment of the device. Silver (1987) discussed the relevant issues, and concluded that intermittent sweep potentials applied to the working electrodes reduced $O_2$ consumption and minimized equilibration time, thereby reducing artefactual influences on tissue and facilitating rapid clinical measurement. With the advent of microfabrication, miniaturized planar $O_2$ electrodes have been produced. In one example, Suzuki et al. (1990) produced a 350µm deep silicon structure with anisotropically etched v-grooves, which contained electrolytes and the surfaces of which included a cathode (working electrode) and an anode. This internal micro-reaction chamber for the polarographic reduction of $O_2$ was bounded by a gas-permeable membrane based on a negative photoresist. The membrane provided for electrochemical stability within the device, protected the sensing surface from biofouling and controlled $O_2$ flux. However, such a Clark-type electrode construction, whilst having advantages for biointerfacing, does complicate micro-device construction and the scale-up of production. A commercially available medical system is marketed by Eppendorf. This microelectrode comprises a recessed gold/platinum wire combination in an insulating glass needle, in turn housed within a 300µm outer diameter protective needle. The recessed gold surface is membrane protected, and so approximates to the desired structure for tissue measurements. Automated smubbimillimetre advance and retraction within tissue allows multiple location measurements, but with post-retraction measurement obviating problems of tissue compression. Tama-Dasu et al. (2001) related the putative $O_2$ consumption of the electrode tip to simulated tissue measurements by differentially weighting $O_2$ at different three-dimensional locations in tissue. They concluded that, for limited measuring times, $O_2$ transport from more distant locations will be restricted, and so what the electrode provides is an ‘averaged’ approximation of the time value of clinical validity, which is not appropriate for data input into more formal tissue modelling procedures. In contrast, direct, free quantitative consumption of $O_2$ by a large electrode applied to the liver surface (Ag working electrode diameter 3 mm) was used intentionally by Seifalian et al. (2000) to measure perfusion driven delivery of $O_2$ to the liver. The electrode had a highly permeable covering membrane. Such a construction could, perhaps, provide a measure of cutaneous circulatory changes.

However, for skin non-invasive monitoring, the conventional approach has been that of the heated transcutaneous $pO_2$ electrode (Huch & Huch 1976). Here, heating permeabilizes the thick outer stratum corneum of skin to allow rapid $O_2$ diffusion. The route taken involves numerous biological barriers, but heating stabilizes and also arterIALIZes the dermal capillary bed. Whilst accuracy is compromised in relation to thicker adult versus neonatal skin, where the electrode has seen most application, Stiecker et al. (2000a) used such an electrode to measure $O_2$ consumption by skin in patients with chronic venous skin lesions. They measured transcutaneous skin $pO_2$ reduction after arresting

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Figure 1. Polarographic ‘stab’ electrode for tissue $pO_2$ monitoring (after van der Kleij & de Koning 1981).
arterial O$_2$ supply and, using the pO$_2$ solubility coefficient for skin, were then able to determine skin O$_2$ consumption, and differences for patients with and without venous ulceration. Evident lowering of transcutaneous pO$_2$ was judged to be a late onset phenomenon.

A more distributive assessment of tissue oxygen, short of a three-dimensional O$_2$ map, is to create a pO$_2$ distribution profile. Using the Eppendorf short penetration/retraction procedure, Nozue et al. (1997) undertook a thorough evaluation of pO$_2$ distribution in defined tumour volumes. There was semi-quantitative agreement between laboratories of the population of tumour hypoxic zones, and such a methodology could also enable more quantitative assessment of skin hypoxia.

### 3. FIBRE-OPTIC O$_2$ SENSORS

Optically based O$_2$ sensors almost all exploit the interference of O$_2$ on the quantum yield and/or lifetime of excited fluorescent or phosphorescent molecules. Specially formulated dyes with high-efficiency O$_2$ quenching have allowed for the fabrication of miniaturized fibre-optic probes able to monitor pO$_2$ over the medically important range (Wolfbeis 1991). The reversible, dynamic quenching occurs through the formation of an oxygen charge-transfer complex on collision of O$_2$ with the excited fluorophor, which in turn leads to non-radiative transfer to the ground state. For the purposes of invasive, transcutaneous monitoring, it is optimum to attach the reagent to the fibre-optic tip. The fibre-optic probe of Peterson & Vurek (1984) translated the principle of O$_2$ quenched fluorescence of a dye to a practical solution (figure 2). The advantage of this approach is that the electrode truly approaches equilibrium, and so does not consume O$_2$ as such; there is no dependence on external tissue flow, and so the device is independent of sample properties. Moreover, there is no external electrical interference. A simple probe without need for a reference electrode (unlike electrochemical devices) is usable. The flexibility of fibre-optics is certainly a disadvantage with respect to implantation, but if, for example, a narrow needle (figure 2) or indeed a retractable needle is used, then direct insertion is possible. The remaining device is a tissue compliant, flexible probe that is less liable to induce pain, tissue irritation or ongoing trauma.

There is now interest in the development of microscale optical probes, e.g. tip diameters in the range 10–30µm. To further create compact systems, the O$_2$ quenched indicator is required to be addressable by a light emitting diode (LED) and a luminescent life-quenched indicator is required to be addressable by fiber-optic probes able to monitor quenching have allowed for the fabrication of minia-}

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Figure 3. Oxygen microelectrode based on a tapered silica-glass fibre fixed in a glass microcapillary (after Holst et al. 1997).

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Figure 2. Fibre-optic O$_2$ sensor based on dye fluorescence quenching exploitation of microporous membrane to retain perylene dibutyrate dye (after Stefansson et al. 1989).
light modulation readily followed by lock-in amplifiers instead of high-speed photodetectors.

For long-term monitoring, photobleaching is a concern, with evidence that, as well as the expected decrease in emission intensity, decay time may also be affected. Hartmann et al. (1997) monitored the contributory effects of singlet oxygen (generated by quenching) on the photobleaching process, and were able to mitigate these through co-incorporation of amine singlet oxygen scavengers.

The principle of fluorescence lifetime decay has been used for skin surface $pO_2$ imaging, on the basis that multiple electrode arrays cannot provide equivalent spatial resolution. Hartmann et al. (1997) developed ruthenium (II) organometallic based $O_2$ permeable films, and topographical lifetime images were obtained that related to spatial $O_2$ distribution on a submicrometre scale using a CCD camera. Transparent planar $O_2$ sensors for such imaging have been developed by Holst & Grunwald (2001), who embedded ruthenium (II)-tris-4, 7-diphenyl-1, 10-phenanthroline with trimethylsilypropanesulphonate as counter ion in PVC coated over transparent polyester. The advantage of the transparent construction is that $pO_2$ distribution can be correlated directly with structural images.

Future optical sensors will have greater stability and biocompatibility. However, improvements in optical characteristics such as excitation/emission maxima, indicator stability, quantum yield and decay profile are likely with new indicators and better polymeric immobilization strategies (Papkovski 1995).

4. CUTANEOUS MICROCIRCULATION

The cutaneous microcirculation has a unique anatomical arrangement that accommodates different and sometimes conflicting functions—the supply of nutrients, clearance of waste products and control of heat exchange (Wheater et al. 1979). The arterial supply and venous drainage to the skin are located deep in the hypodermis. They give rise to arterioles and venules to form two important plexuses in the dermis: the deeper cutaneous plexus at the junction of the hypodermis and the dermis and the superficial sub-papillary plexus just beneath dermal papillae (Braverman 1997). The sub-papillary plexus supplies the upper layer of the dermis and gives rise to a capillary loop in each dermal papilla. In the epidermis, cells produced by mitosis in the germinal layer undergo different stages of maturation and move progressively to the outer layers. The cornified layer in the outer most region of the skin consists of flattened cell remnants that are constantly shed (figure 4).

The superficial layer of the skin is the region between the sub-papillary plexus and the surface of the skin. While there is recent evidence that $O_2$ entering the skin from the atmosphere may meet the oxygen requirements of the cells in these areas (Stücker et al. 2002a), counter-current arranged capillary loops in the papilla and their significance to oxygen delivery to the epidermis has not be investigated (Wang et al. 1996; Wang 2000). While there have been many studies on mechanisms of oxygen transport within tissues including the skin (see the reviews by Scheuplein & Blank (1971), Popel (1989) & Pittman (1995)), much of the experimental work on skin has been limited to estimates of mean tissue $pO_2$ from measurements of transcutaneous $pO_2$ or has used needle electrodes that were large relative to the dermal papilla (e.g. Evans & Naylor 1966; Spence & Walker 1976; Lübbers 1987; Opitz & Lübbers 1987; Jaszczyk 1991; Harrison et al. 2002). There have been few attempts to relate the spatial distribution of $pO_2$ to the arrangement of the cutaneous microcirculation using small oxygen sensitive microelectrodes (Wang et al. 2003).

5. MICROELECTRODES FOR $pO_2$ IN HUMAN SKIN

In studies using small microelectrodes, $pO_2$ measurements are limited to the superficial layers of the skin,
since the penetration of deeper skin and underlying muscle requires much stronger, hence bigger electrodes. Oxygen sensitive microelectrodes were made using platinum–iridium wire. A short length of 25 μm diameter Pt–Ir wire (Goodfellow, Cambridge) was electrochemically etched to a slender profile with a tip diameter of less than 1 μm in saturated nitric acid solution. It was soldered to a conducting wire and was then fixed inside a 1.5 mm standard-walled glass pipette (Clark, Reading) using epoxy resin Araldite (Winlove & O’Hare 1993). The glass pipette was mounted on a micropipette puller (Narishige, Japan), which pulled the electrode to the desired tip size and profile. The pulling conditions were adjusted so that the glass sealed around the top of the wire. The tip of the electrode was bevelled using a micropipette grinder (Narishige, Japan) to enable easy tissue penetration. The tip of the finished electrodes was approximately 3–8 μm in diameter, giving a theoretical spatial resolution of 20–50 μm. The surface of the metal electrodes was cleaned by sonication in distilled deionized water and was coated with Nafion perfluorinated ion exchange resin (Sigma–Aldrich, Dorset).

To investigate the effects of occluding the local microcirculation on the tissue pO2, some electrodes were made with special φ-shaped tips by repeated heating of the tip of the pulled electrode using the heating element of the micropipette puller and fusing a small glass bead to the shaft approximately 200 μm above the tip. These microelectrodes behaved identically to conventional electrodes as they penetrated the tissue up to the depth at which the bulbous region of the glass shield came into contact with the skin surface. Further advance of the electrode now compressed the underlying tissue, occluding the local microcirculation. This provided a convenient method for investigating the transient effects of microcirculatory arrest upon tissue pO2.

All electrodes were tested for their electrochemical characteristics and were kept in saline for storage before and after testing and measurements. Calibration of electrodes was carried out before and after each experiment in saline bubbled with 100% N2, air and 97% O2 + 3% CO2 to provide pO2 values at atmospheric pressure. It is not uncommon for electrodes of this size to break during experiments. The most common cause of these accidents was unconscious movements of the finger during experiments. Penetration of skin and advance of electrodes in tissue were also occasionally responsible. If an electrode broke while in the tissue, the area was viewed under the microscope and any broken remnants of electrode were removed. The stratum corneum was removed for easier electrode penetration using adhesive tape before the start of an experiment. The lower arm and hand of the subject were taped on to a steel plate on the table to minimize excessive movement of the hand. The reference electrode (Ag/AgCl ECG electrode) was placed on the skin near the finger. The microelectrode was mounted on a three-dimensional remote hydraulic micromanipulator (Narishige, Japan), which in turn was mounted on a three-axial coarse micromanipulator (Narishige, Japan). Both the working and reference electrodes were connected to the potentiostat (CV37, BAS). Currents from the potentiostat at −0.75 V versus Ag/AgCl were recorded on a chart recorder.

Skin was illuminated using a cool light source. Paraffin oil was super fused over the finger to minimize the transport of oxygen from the air and to reduce light reflections from the surface of the skin. A Wild M10 stereomicroscope (Leica) was used to visualize nail fold capillaries and flows in these capillaries. No measurements of blood-flow velocity were made, but red cells were used as markers to indicate flow conditions (e.g. flow stopped, partially stopped, reinstated). The coarse micromanipulator was used to position an electrode just above the site of the measurement, while the remote hydraulic micromanipulator was used for the penetration of the skin and movement of the electrode within the tissue.

In all measurements, the oxygen partial pressure of the air is 160 mmHg at sea level and at room temperature.

6. SPATIAL VARIATIONS OF pO2 IN SUPERFACIAL LAYERS OF SKIN

Mean values for the spatial variations of the pO2 with depth in the outer skin are presented in figure 5. In figure 5a, mean pO2 (±SD) has been plotted for the superficial (5–10 μm), the middle (45–65 μm) and deep (100–120 μm) regions in the dermal papilla of finger nail folds. It is seen that the pO2 increases with depth from the surface of the skin to the dermis. With paraffin oil applied on top of the nail fold, pO2 was lowest at the surface of the skin. In the superficial region of the skin, pO2 is approximately 8.0 ± 3.2 mmHg (n = 6). The value increases to 24.0 ± 6.4 mmHg (n = 8) in dermal papillae, and at the depth just above the sub-papillary plexus, pO2 is approximately 35.2 ± 8.0 mmHg (n = 9). Student’s t-test showed that these results differed very significantly: between the superficial and the middle regions to a level of p < 0.00005, and between the middle and the deep regions to a level of p < 0.005.

pO2 along the axis of papillary loops was measured, mean pO2 (±SD). These measurements were carried out with the microelectrode tip at points in the tissue that were close to the most distal (and hence most horizontal) capillary loops of the nail fold. As shown in figure 5b, pO2 generally decreased from the base to the tip of the papillary loop. At the base of the loop, pO2 is approximately 40 ± 4.8 mmHg (n = 6), the value drops to 35.2 ± 3.2 mmHg in the middle (n = 6) and approximately 30.4 ± 5.2 mmHg near the tip of the loop (n = 6). Student’s t-test revealed that p < 0.01 between data at the base and the tip of the papillary loop; however, the differences between values at the base and the middle of the papillary loop or between the middle and the tip of the loop are not significant (i.e. p < 0.13 and p < 0.10, respectively).

7. TEMPORAL VARIATIONS OF pO2 FOLLOWING MICRO-OCCLUSION

Tissue oxygen content is the balance between the oxygen supply and oxygen consumption. To gain insight into this, special φ-shaped electrodes were used to
investigate the transient changes in skin $pO_2$ when the microcirculation is suddenly arrested and restored.

In experiments where flow in the capillary and underlying sub-papillary plexus was either reduced or stopped, $pO_2$ in dermis decreased with the time in all cases by 16–24 mmHg. New steady values in $pO_2$ were reached within 30–60 seconds following alterations in capillary flow. The dynamic changes in $pO_2$ with the time can be described by a single exponential decay function,

$$P = a \exp(-t/\tau) + b,$$

where $P$ is the value of the $pO_2$ during the transient, $\tau$ is the time constant, $a$ represents the difference between the initial and final values of $pO_2$ and $b$ is the final value of $pO_2$.

Figure 5. Spatial variations of $pO_2$ in the outer layers of the nail fold skin (from Wang et al. 2003). (a) Mean values for $pO_2$ at depths of 5–10 μm (surface), 45–65 μm (middle) and 100–120 μm (deep) in all areas of the nail fold skin. The $pO_2$ at the surface and in the deep regions differ very significantly from those in the middle region ($p<0.0005$ and $p<0.005$, respectively). (b) Mean values for $pO_2$ with depth in tissue close to the axis of the capillary loops of the papillae. While $pO_2$ at the tip differs significantly from its value at the base ($p<0.01$), the gradient is too small for significant differences to be detected between the $pO_2$ of the tissues around the middle of the capillary loops and the $pO_2$ at the tip and the base.

After a period of microcirculatory arrest lasting between 1 and 5 minutes, flow was restored. The increase in $pO_2$ can be described by a similar single exponential function,

$$P = a'[1-\exp(-t/\tau')] + b',$$

with $\tau'$ being the time constant for the exponential rise, and $a'$ and $b'$ representing the range and initial value of $pO_2$.

Time constants (mean ± SD) for the exponential decays (ischaemia) and rises (reperfusion) of the $pO_2$ are shown in figure 6a. It was found that the mean time constant for decays, $\tau = 8.44 \pm 1.53$ s ($n=7$), is consistently greater than that for the exponential rises, $\tau' = 4.75 \pm 0.82$ s ($n=6$). A two-tailed $t$-test was applied to the data and revealed a significant difference between the time constants ($p<0.0005$).

In figure 6b, steady values of the $pO_2$ (mean ± SD) following ischaemia and after reperfusion were compared with their values prior to arrest of the microcirculation (the control state). There is a significant decrease
in the pO₂ following arrest of the local microcirculation. pO₂ falls from a mean of 35.2 ± 4.8 mmHg (n = 6) to 19.2 ± 6.4 mmHg (n = 6), i.e. a fall in pO₂ of 16 mmHg. The non-zero value for pO₂ following tissue compression is caused by either the partial occlusion of the circulation in some experiments or O₂ supply from neighbouring vessels or both. When blood flow is restored pO₂ increases to 43.2 ± 6.4 mmHg (n = 6), an increase of approximately 125% in comparison with the value in ischaemia. This value, interestingly, is also 23% greater than the control. Student’s t-test revealed that differences in pO₂ between ischaemia and control, or between ischaemia and reperfusion were highly significant (p < 0.0003 and p < 0.0004, respectively). There was also significant difference between pO₂ values in control and in reperfusion, p < 0.05.

8. O₂ SUPPLY TO SKIN

The study using small microelectrodes showed that when the skin of the human finger nail fold is covered with paraffin oil, the pO₂ in the tissue increases with depth from values close to zero at the surface to about 40–50 mmHg close to the sub-papillary plexus. These agree with previous experimental and theoretical estimates of pO₂ in this part of the skin (e.g. Grossmann 1982; Jaszczak, 1991; Baumgärtl et al. 1987; Sticker et al. 2000b). The observation that pO₂ increases with depth would be predicted if all O₂ is delivered to the papillary dermis and epidermis from the sub-papillary plexus. Under physiological conditions, however, O₂ can enter the outermost layers of the skin from the atmosphere and recent measurements of Sticker et al. (2002) have shown this may be as much as 5.3 ml cm⁻² min⁻¹ and would be sufficient to meet the needs of the epidermis. Under these conditions the pO₂ profile with depth beneath the skin surface might be expected to differ considerably from that reported by Wang et al. (2003). Although there is now strong evidence that O₂ may enter the skin from the atmosphere, the study by Wang et al. (2003) demonstrated that when this is prevented, the oxygen supply to the epidermis and superficial dermis can be maintained by the papillary microcirculation. There is also a suggestion that the papillary microcirculation is regulated to meet the O₂ requirements of the tissue at a very local level.

9. EFFECTS OF LOCALIZED ISCHAEMIA REPERFUSION

The exponential decline in pO₂ when flow is stopped is most likely to be caused by consumption of the oxygen in tissue and the blood, and would be consistent with the discharge of O₂ from stores in the tissue. The most likely site of these O₂ stores is the haemoglobin of the red cells in the capillaries where flow has been arrested. The oxyhaemoglobin dissociation curve is approximately linear over the range of pO₂ between 40 and 15 mmHg, and the unloading of O₂ over this range might be expected to approximate to a single exponential function.

The rise of pO₂ to a new steady state as flow is restored is determined by the rate at which the pO₂ in capillaries is returned to their pre-occlusion values. This is primarily dependent on the blood flow velocity in the re-perfused capillaries. If the tissue at the tip of the microelectrode is principally supplied by O₂ from a point along a nearby capillary, the rate at which capillary pO₂ will rise at this point will depend on how rapidly blood with a higher pO₂ reaches that point and how much the blood pO₂ falls between leaving the arteries and arriving at this point. Provided that the O₂ consumption of the tissue remains constant, the extraction (i.e. the O₂ lost from unit volume of blood) should be largely dependent on the blood flow. If the post-occlusion flow is greater than in the pre-occlusion state, not only will the pO₂ rise rapidly, but it should rise to a higher level than it was initially. The tissue pO₂ should then remain above its initial value for as long as the flow is elevated. This is just what has been observed. When blood flow is restored, tissue pO₂ rises to a value that is 23% higher than its pre-occlusion value. This is consistent with either a decrease in tissue O₂ consumption or a significant degree of reactive hyperaemia. Assuming that tissue O₂ consumption is the same as in the pre-occlusion state, the 23% increase in pO₂ is equivalent to an increase in O₂ saturation in the blood of the nearest capillaries from 70 to 85%.

If the saturation of the arterial blood is 95%, the increase in blood flow equivalent to the rise in tissue pO₂ is of the order of 2.5-fold. This calculation indicates that reactive hyperaemia occurs with a high spatial resolution in skin and this would be consistent with the very localized hyperaemic responses seen in other tissues (Burton & Johnson 1972). Although the rise in pO₂ accompanying reperfusion can be described approximately by a single exponential, the data are less regular than those describing the decline on pO₂ with vascular occlusion. This may represent fluctuations in blood-flow velocity or red cell flux with the restoration in flow (Johnson & Wayland 1967).

10. FUTURE PROSPECTS

The electrochemical and fibre-optic devices described in this section are best regarded as semi-implantable and minimally invasive. They have the potential to provide localized information of (at least) approximate pO₂ in pre-specified skin and skin ulcer microenvironments. There are two major developments that are likely to improve spatial discrimination and reliability. First, evolving microfabrication techniques will allow robust probe structures to be fabricated as arrays able to penetrate in and around the ulcer wound site, but with negligible local trauma. This “atraumatic” insertion should minimize local tissue reaction with less tissue disruption. Devices will then become truly minimally invasive and yet have a high sensor/interrogation density, almost with the features of a chemically reactive velcro. A further advantage will be of a high level of sensor redundancy, allowing for greater confidence in the measurements. With appropriate additional hollow microneedle and microinjection capability along such arrays, it could be envisaged that pharmacologically active agents might be injected locally, e.g. vasodilators to monitor the reactivity of localized circulation as
a possible dynamic indicator of tissue integrity. The second key advance will come from materials science. Membrane technology has already improved the stability and biocompatibility of biosensors. Further advances should help to reduce the inevitable drift that O$_2$ sensors suffer due to surface fouling and contamination (Reddy & Vadgama 1997).

Other possible advances will exploit skin ulcer tissue embedded optical nanoprobes coated with, say, O$_2$ reactive dyes, so developing the recent concept of probes encapsulated by biologically localized embedding, so called PEBBLE sensors (Xu et al. 2001). Whilst such micron scale spheroids have allowed distributed sensing in vitro, the correct formulation may enable safe in vivo use and, moreover, with near-infrared active dyes, interrogation of deeper skin layers becomes possible. The ultimate goal must be sensor-based feedback on a real-time basis so that local pO$_2$, a key surrogate of tissue metabolic status, can be used to optimize pressure ulcer therapeutic regimens.

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