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Novel flexible enzyme laminate-based sensor for analysis of lactate in sweat

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ABSTRACT

We present work towards a novel amperometric enzyme-based highly flexible biosensor for real-time and non-invasive monitoring of lactate in human sweat for the early detection of pressure ischemia onset. The core of the recognition system is a highly flexible laminate, comprising two highly porous polycarbonate membranes, which provide support for the lactate oxidase enzyme (LOD), immobilised via covalent crosslinking. A number of variables were assessed to attempt to optimise the sensors, such as membrane pore size, crosslinking time, crosslinking agent concentration and levels of incorporated enzyme. Oxidation of lactate produces H_2O_2, which is subsequently determined electrochemically. The transducer comprises a two-electrode system on a single highly flexible polycarbonate membrane, sputter-coated with gold and platinum to render it conductive. The sensor exhibits lactate selectivity with a working range of 0–70 mM, thus covering physiologically relevant concentrations for pressure ischemia and has been shown to be suitable for determination of lactate in PBS, synthetic sweat and diluted human sweat.

1. Introduction

Pressure ischemia is the condition in which skin and underlying tissue necrosis occurs due to malnutrition of the tissues in body areas exposed to continued pressure, or pressure in combination with shear and/or friction [1].

Pressure ischemia has significant health, quality of service and economical implications. It is estimated that, in general terms, approximately 9% of hospitalised patients develop pressure sores [2]. People more susceptible to pressure ulcers include those with mobility problems such as bedridden, wheelchair-bound, debilitated or paralyzed patients. People suffering from cardiovascular and neurological diseases can in many cases also suffer from pressure ischemia, since these diseases lead to impairment in circulation [1]. Moreover, it is well understood that pressure ischemia is related to a notable increase in mortality rates [3]. In acute care hospitals, the prevalence of pressure ulcers is approximately 10% [4]. It is estimated that the treatment of pressure ulcers costs the UK National Health Service 4% of its budget, a cost of between £1.4 and £2.1 billion per year [5]. It is for this reason that pressure ischemia has significant implications in cost and quality of service for the health sector. However, pressure ischemia is considered a preventable condition that arises mainly from poor clinical management and, more importantly, a lack of warning indicators [6].

When the skin undergoes prolonged pressure, the underlying blood vessels become occluded, either partially or totally. As a result of this, oxygen and other nutrients carried in the blood are not delivered in sufficient quantities to satisfy the metabolic demands of the affected tissue. Cells are then obliged to use their own stores of energy through an anaerobic metabolic pathway in order to survive, leading to the production of more lactic acid, which begins accumulating [6] within both the affected cells and the interstitial spaces. As the levels of energy stores diminish, cellular processes start to fail, the ionic flow across cellular membranes begins to fall and lactic acidosis can occur. These processes can lead to cell death [7,8] and cell necrosis occurs, with the subsequent formation of a pressure ulcer (pressure sore). Hence, pressure ulcers arise from prolonged tissue ischemia [5]. For this reason, lactate levels are a

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useful alarm signal for the diagnosis of patient conditions in general clinical practice as well as in hospital intensive care units and operating rooms.

One of the most common clinical practices for the early detection of pressure ischemia was based on a periodical inspection of the skin colour of the patient. Later diagnosis methods were based on an alternative approach using tissues as the source of information. The first technique consisted of the monitoring of blood flow, which constituted a slight improvement from the observation of skin colour [9]. It was known that even in early stages of pressure ulcer formation, the tissues responded with an accumulation of metabolites, a decrease in pH and oxygen partial pressure (PO_2, which is the amount of oxygen that will bind haemoglobin within the red blood cells) and an increase in partial pressure of carbon dioxide (PCO_2) [10]. However, the techniques derived from this idea implied the use of invasive methods on areas of risk for the collection of samples. Pressure ischemia is considered a preventable condition that arises from poor clinical management, reduced nursing staff and a lack of warning indicators [6]. However, in reality it is understood that it is not that easy to prevent because of the many factors that cause it.

Lactate levels constitute a useful warning indicator for the formation of pressure ulcers. In susceptible patients, pressure ulcers can develop in just one or two hours [11], even though in some cases the damage will only become evident a few days later. One approach would be to use biosensors to give direct, simple and highly specific lactate measurements, low response time, inexpensive and with minimal or no sample preparation. Commercially available biosensors for blood lactate have been developed but require an invasive method of sample collection or, in the case of implantable vascular sensors for in-situ monitoring impart a high risk of thrombosis [12]. This issue and the current trend for non-invasive diagnostic and real-time monitoring methods have led researchers to search for other body fluids on which to perform lactate measurements and among these, sweat shows the most promise, since it is the most accessible specimen to collect, its collection is non-invasive and its real-time analysis offers valuable physiological information [13]. Also, sweat analysis allows specific body areas to be studied (i.e. pressure points).

It was suggested that sweat lactate would be a good marker for evaluating the severity of peripheral occlusive arterial disease which implies a local occlusion of blood supply [14]. Subsequent studies have suggested that sweat lactate, produced as a by-product of the anaerobic metabolism of glucose by the eccrine sweat glands, could be a sensitive indicator of damage in soft tissues [15–17].

Normal values of lactate in human perspiration are 20 ± 7 mM, but under ischemic conditions these can rise up to 62 ± 16.3 mM [17]. In order to achieve continuous monitoring, wearable sensors have attracted increasing interest from the research community and industry during the last decade [18]. To date, most of these sensors have been developed for the monitoring of physiological parameters of the patient, such as breathing rate, heart rate or temperature [13]. Wearable chemosensors, although at an early stage, have the potential to measure many more variables related to the wearer's health. Moreover, the potential of these sensors to perform chemical and continuous measurements of body fluids (i.e. sweat) opens a new insight for medical science by providing valuable real-time feedback information about the patient's health status, which is the key for preventative healthcare and early diagnosis of conditions such as pressure ischemia.

A review of lactate biosensors is beyond the scope of this paper, however previous workers [19] have analysed the different aspects within the preparation of amperometric lactate biosensors such as biorecognition elements, methods of immobilisation, mediators and cofactors, and fields in which these sensors can be applied. Most of the sensors utilise the technology developed for glucose monitoring, with lactate enzymes such as lactate oxidase (LOD) forming the active biorecognition element. Flexibility would be a big advantage to these biosensors since it would enable them to be worn as they could conform to the shape of the human body. There has also been other work on lactate monitoring during exercise, these tend however to monitor blood lactate and show that it peaks during and just after intense exercise [20,21]. However lactate in sweat has been monitored; sweating was induced by heating or exercise and then tested using biosensors based on a Rank oxygen electrode and membranes containing lactate oxidase or ion selective electrodes [22], lactate levels of up to 115 mM were obtained and it was claimed that the analysis of lactate in sweat is an effective, non-invasive and convenient method for estimation of physical condition.

A flexible system based on platinum microdisc electrodes on a polyimide substrate has been developed [23], the resulting strip being used to immobilise glutamate oxidase and lactate oxidase to give a biosensor for glutamate and lactate., although response of the lactate sensor was highly oxygen limited. Other workers [24] developed a biosensor that could be applied as a "tattoo". Lactate-oxidase functionalised carbon electrodes along with reference and counter electrodes were printed onto a paper substrate; this was then use to transfer the "tattoo" onto a subjects deltoid. Production of lactate upon exercise could be measured, the biosensor had a linear current range up to 20 mM lactate and was stable to deformation resulting from wear [24]. Disposable lactate biosensors have been developed based on platinum decorated carbon nanofibres, these displayed sensitivity of $36.8\ mA\ M^{-1}\ cm^{-2}$ with a linear range of 25–1500 μM lactate, limit of detection 11 uM [25].

Our present work describes the development towards a novel flexible biosensor for real-time, continuous and non-invasive sensing of lactate in human sweat. We attempted to optimise the performance of the sensor so that its active range would be suitable for the monitoring swear lactate at levels found in healthy sweat and that from ischemia patients. We therefore varied the enzyme loading and crosslinking time of the active membrane, pore size of supporting membrane and developed the use of a flexible support. This potentially will lead to development of an external sensor that will be directly applied to the skin of patients as a real-time, non-invasive test for the onset of pressure ischemia. It would also have potential in the fields of sport science.

2. Methods and materials

2.1. Reagents and materials

Ferrocene carboxylic acid, lactate oxidase (from Pediococcussp, lyophilised powder, ≥20 units/mg solid), sodium L-lactate and bovine serum albumin (BSA, lyophilised powder) were purchased from Sigma-Aldrich (Dorset, U.K.). Sodium dihydrogen orthophosphate 1-hydrate ($NaH_2PO_4{\bullet}H_2O$), disodium hydrogen orthophosphate 12-hydrate ($Na_2HPO_4{\cdot}12H_2O$), sodium chloride, urea, ascorbic acid, glacial acetic acid, hydrochloric acid and ≈50% glutaraldehyde solution were purchased from VWR BDH (Poole, U.K.). Uric acid was purchased from Acros Organics (Loughborough, U.K). All reagents were used as received unless otherwise stated.

Deionised water for preparation of all solutions was obtained using a Purelab® UHQ system from ELGA (Marlow, U.K) and had a resistivity >18 $M\Omega\ cm^{-1}$. A phosphate buffered saline (PBS) solution was prepared at pH 7.4 containing 5.28×10^{-2} M $Na_2HPO_4{\cdot}12H_2O$, 1.3×10^{-2} M $NaH_2PO_4{\bullet}H_2O$ and 5.1×10^{-3} M NaCl. BSA solutions were prepared at a concentration of $0.1\ g\ mL^{-1}$ in PBS. A lactate oxidase/BSA solution was prepared using LOD at the desired concentration dissolved in $0.1\ g\ mL^{-1}$ of BSA solution