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SMART WEARABLE BIOSENSOR FOR NONINVASIVE REAL-TIME DETECTION OF SWEAT LACTATE USING COMPRESSION GARMENTS

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ABSTRACT

Over the past decade, there had been a surge in the use of wearable sensors to monitor health specially to determine the individual's fitness level. It has been reported that lactic acid is a significant biomarker of anaerobic metabolism and higher concentrations of lactate in sweat can cause Ischemia and lead to hypoxia. Although, there had been an increase in the use of smart wearables such as heart rate, blood pressure, skin pH, and so forth, very little had been reported on the use of body fluids such as sweat. Therefore, a non-invasive monitoring of blood lactate becomes essential in determining individual's health and fitness.

In this research, the development, characterization and optimization of an electrochemical-based amperometric lactate biosensor screen-printed on to a knitted fabric is reported. The prototype screen-printed fabric lactate biosensor is composed of three electrodes that senses lactate concentration from the body sweat collected. A highly sensitive and stable lactate sensor based on PEDOT: PSS/PVA has been developed. The research will use wearer trials wearing prototype compression garments and measurements such as blood lactate, sweat rate, and garment performance in the subsequent stages of the research. The information obtained from this study will inform the design and development of compression garments that enhances blood flow, increases oxygen delivery to the muscles, and reduces the blood lactate concentration. The wearable device will also enable athletes to monitor their real time lactate concentration and pace their activity.

Keywords: Biosensor, lactic acid, blood lactate concentration, electro-chemical biosensor, knitted fabric, and compression garment.

INTRODUCTION

Compression garments for sportswear and leisure applications have become widely used, providing increased comfort, fit and muscle support (Venkatraman and Tyler, 2016). The application of compression garment to support and apply pressure to muscles as a recovery tool to reduce post–exercise trauma and perceived muscle soreness have been documented (Duffield and Portus, 2007). Compression shorts improved repetitive vertical jumping performance of volleyball players (Kraemer et al., 1996). The reported **benefits of**

compression wear in rugby include: reduce muscle soreness and swelling; reduce muscle oscillation during a vertical jump or fall; increase VO_2 max [maximum oxygen uptake, a physiological index of sports performance]; reduce collection of blood lactate levels in the tissue and reduce muscle injury or cramps (Venkatraman and Tyler, 2016). The use of lower and upper body compression in exhaustive exercise improved exercise tolerance and reduced blood lactate levels after exercise (Lambert, Duffy and Chow, 2004). Reports indicated that wearing compression garments during exercise reduced micro-trauma and muscular damage (Trenell et al., 2006). Compression garments compress the body through the pressure applied to the skin and musculature and it depends on the mechanical properties of the garment (McRae, Cotter and Laing, 2011).

Lactic acid is produced during physical activity; this occurs when increasing amount of energy is produced anaerobically. In other words, glucose is not broken down to water and carbon dioxide due to lack of sufficient oxygen at the site of reaction (Reilly, 1990). Lovell et al., (2011) investigated whether compression garments enhance active recovery process after high intensity running (30-minute treadmill run) using 25 semi-professional rugby league players. Findings revealed that wearing compression garments might enhance recovery process in reducing blood lactate and heart rate after high intensity.

During high intensity exercise, muscles produce large amount of lactic acid, once produced lactic acid rapidly ionises by releasing hydrogen ion, the remaining molecule is called **lactate**. During physical activity, the oxygen uptake increases in a linear fashion during incremental exercise until **VO_2 max** is reached. The point at which blood lactic acid rise systematically during graded exercise is termed as **lactate threshold**. Lactic acid is produced due to the combination of: (1) low muscle oxygen; (2) reduced rate of lactate removal; (3) accelerated glycolysis and (4) recruitment of fast fibres (Powers and Howley, 2007). Lactate threshold used in conjunction with VO_2 max is a predictor of sports performance in long distance athletics or endurance activity and helps in planning training programme. In sports, blood lactate levels during exercise are an indicator of training status and fitness (Nikolaus and Strehlitz, (2008) and Cai et al. (2010)). It is estimated that 70% of lactic acid produced during exercise is oxidised, 20% is converted to glucose and 10% converted to amino acids. It was also reported that removal of lactic acid during continuous light exercise is more rapid than no exercise, due to the fact that oxidation of lactic acid by the working muscle (Powers and Howley, 2007).

Formation of sweat is a natural biological process and is produced to keep the body cool and prevent from overheating, during a physical activity or exercise. Smith and Havenith (2011) investigated the sweat patterns of nine male Caucasian athletes during exercise in warm conditions. They reported that sweat rate increased significantly with exercise intensity in all regions except the feet and ankle. Lower back (posterior torso) consistently showed the highest sweat rates over the whole body. Highest **sweat rates** were observed on the central and lower back (posterior torso) and forehead whilst the lowest values were observed towards the extremities. Sweat is a clear hypotonic, odourless fluid containing sodium chloride, potassium, urea, lactate, bicarbonate, calcium, ammonia and non-organic compounds (Whitehouse, 1935). It has been noted that heat, mental stimuli, muscular exercise and carbon dioxide will induce active sweating in humans (Ohhashi, 1998 and Bullard, 1964). Morris et al. (2009) recently reported monitoring of sweat on a real time basis using a bio-sensing textile based patch with an integrated optical detection system. The researchers reported that such sensors would find applications in personal health and sports performance and training. The investigators highlighted that the fluid handling system is

complicated and their work was based on using a textile based sample collector. They also reported pH sensor based on emitter-detector LEDs and it was noted that an increase in sweat pH during exercise. Baysal et al. (2014) reported the development of nonwoven based biosensor for sweat analysis. The researchers used photolithography technique on nonwoven fabric to fabricate patterns comprising of channel system to transfer the simulated sweat for analyte detection. An electro-chemical based tattoo biosensor was reported that monitors lactate during exercise (Jia et al. 2013).

A **bio-sensor** is an artificial device that is constructed with a biological recognition element (enzymes, tissues, etc.) and a transducer (such as electrode, carbon nano-tubes) to detect the presence of chemicals. It converts biological response into electrical signals which can be readily measured and quantified. Cai et al., (2010) reported the use of electrochemiluminescent (ECL-based biosensor) that was practical in detecting the lactate from sweat samples of athletes during exercise. Weber et al., (2006) reported the use of carbon nano-tubes in monitoring pH and lactate from sweat using a single walled carbon nano-tube. Matzeu (2015) reviewed various wearable chemical sensors for monitoring biological fluids.

Formation of blood lactate during intensive physical activity especially when the muscles do not receive oxygenated blood affects the performance of an athlete. Hence, it is a **physiological marker** to determine the performance of athlete (Performance Apparel Markets, 2014). Compression garments have been marketed by industry to promote blood circulation and removal of blood lactate formation by applying mechanical pressure to the tissues. Bio-sensors have been used in food and beverages and cancer detection and is expected to grow and reach $21.6 Billion by 2020 from $12.4 in 2013 (Transparencymarketresearch.com, 2015). Previous researches have not highlighted the application of bio-sensors in garments. This study will aim to develop biosensors that can be embedded in compression garments to monitor blood lactate and further the current knowledge of non-invasive monitoring of blood lactate concentrations. This real time blood lactate assessment will enable trainers to monitor the performance of athletes and device a suitable training programme.

Methodology

To date blood samples (using a finger prick) are collected from participants to detect the lactate levels using a lactate plus meter. However, in this project biosensors are proposed that detects the blood lactate formation using body sweat which is a non-invasive method. In this project, researchers conducted experiments in the laboratory to identify a specific electro-chemical based amperometric lactate biosensor. The prototype lactate biosensor was composed of three electrodes characterised and optimised to determine blood lactate from body sweat. The project had obtained ethical approval from the Head of Ethics, Science and Engineering, Manchester Metropolitan University and experiments were conducted in compliance with the University regulations.

Biosensor positioning: Data collated by Havenith et al., (2007) identified the highest sweat rate on the male body as the mid lateral back, along the spine and specifically in the lower back (Havenith et al., 2007). It was therefore decided to locate the biosensor within this

region in order to collect the optimum amount of sweat for analysis (Figure 1). The biosensors are positioned under the arms, chest region and in the lower back.

Garment design and finish: A full sleeve compression garment (CG) with crew neck has been developed, ensuring maximum skin coverage and offering support to all muscles within the upper body. Wearing CGs results in higher skin temperatures (MacRae, Cotter and Laing, 2011) and therefore provides the optimal environment for collection of sweat samples. As a first layer garment, in direct contact with the wearer's skin, the CG will utilise stitch less manufacturing processes, substituting traditional stitching for bonded finishes, ensuring a smooth finish and reducing seam irritation. Bonding replaces traditional stitching within sportswear garments, making close fitting garments even more streamlined (Shishoo, 2005).
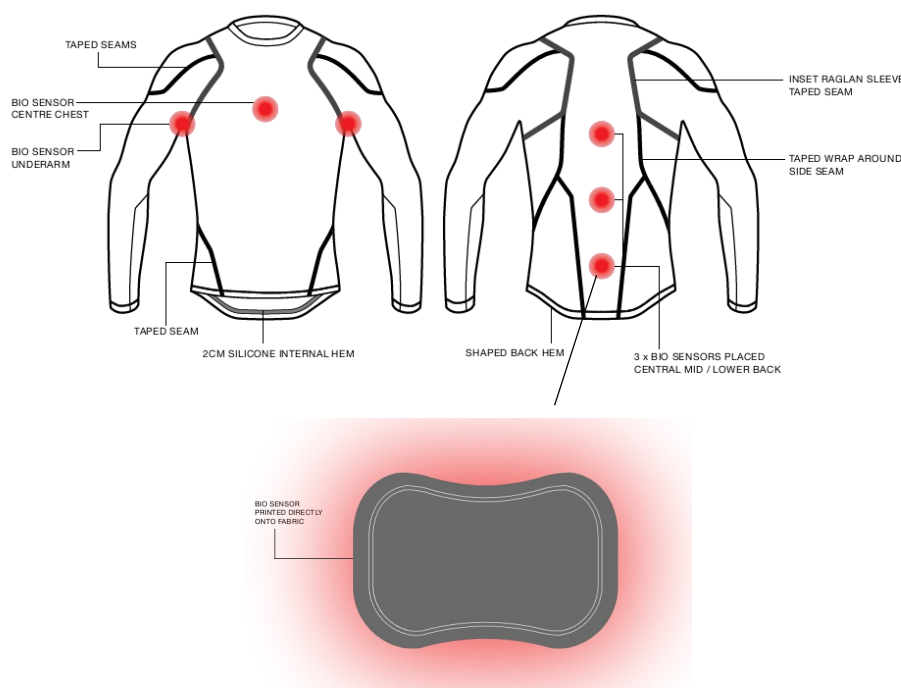
Figure 1 Biosensor and prototype compression garment with biosensor positioning

The key features of the CG have been developed primarily to aid wearer comfort and ensure the CG is fitted closely to the body, some consideration of aesthetic appeal has however also played a role within the development process. The raglan armholes are angularly shaped into the main body, allowing increased freedom of movement by the wearer and reducing irritation from the sleeve head on shoulders and under arm. Similarly, the standard side seam has been replaced with an ergonomic seam that wraps around the body, mapping the natural movement of the wearer, again this seam will be heat sealed/bonded to reduce irritation or chaffing. Taped seams are also used to provide water repellent and durable outlook and at the same time to offer the ability to accumulate sweat within the garment.

As there are large variations in sweat rates at various parts of the body it is necessary to stabilise the position of the biosensor. The back hem of the CG has therefore been lowered and shaped to house an internal 2 cm strip of silicone. The silicone will act as a gripper, helping to ensure the garment and the biosensor will remain in a more constant position throughout the trials.

Fabrication of Lactate Biosensor

Amperometric biosensor exploits the current produced when an oxidation or reduction reaction occurs at an electrode; current response generated during the reaction is directly proportional to concentration of that species present in the particular sample. Therefore, it functions by the measurement of the current produced on application of potential between working and reference electrodes that results with the electrocatalytic oxidation or reduction of the involved electroactive species. The magnitude of this current is directly correlated to the concentration of a redox-active reagent or product in an enzymatic reaction. Amperometric biosensors offer a sensitive and selective detection to monitor organic analytes such as lactate. Among the various lactate detection methods, enzyme based electrochemical lactate sensors are of the predominant type due to their sensitivity, low detection limits, comparatively simple fabrication, user friendly, portability, reliability and cost effectiveness. In the fabrication of lactate biosensors, the most commonly used biological recognition element are lactate dehydrogenase(LDH) and lactate oxidase(LOD) due to prevalence of simple enzymatic reaction involved and considerably simple sensor design fabrication.

Conducting polymer matrices based lactate biosensors biocompatible in neutral aqueous environment, enhances the sensitivity and selectivity as a result of good electrical conductivity and their electron transfer channel. A well-studied and widely used conducting polymer is poly(3,4-ethylenedioxythiophene) doped with poly styrene sulfonate (PEDOT: PSS), a p-type conducting polymer in which the negative charge of PSS is compensated by a hole in the PEDOT backbone. This conducting polymer exhibits high electronic conductivities, with typical conductivity values of commercially available PEDOT: PSS reaching approximately 1000 Siemens per cm (S/cm). PEDOT: PSS, has electrical properties superior to those of most conducting polymers, but it is too brittle to be employed as fabric sensor. Blending PEDOT: PSS with other polymers is a promising route to reach a good trade-off between electrical and mechanical properties. While the tensile strength of pure PEDOT: PSS fibre was low, it was unsuitable to fabricate textile-based sensors. To avoid this drawback, we examined the composite fibres composed of PEDOT: PSS and poly vinyl alcohol (PVA) to obtain good mechanical properties as well as a high electronic conductivity. PVA was used as a matrix component to connect colloidal PEDOT: PSS particles within the fibres. We fabricated conductive fibre made from organic conjugated polymer composites fibres composed of PEDOT: PSS and PVA to obtain good mechanical properties as well as a high electronic conductivity. PVA was used as a matrix component to connect colloidal PEDOT: PSS particles within the fibres. Textiles made of conductive fibres behaved as flexible electrodes for the detection of lactate.

In this study, PVA was blended with PEDOT: PSS in order to enable the formation of free-standing films with a well-balanced combination of strength and ductility. PVA, a water soluble synthetic polymer, has excellent film-forming, emulsifying, and adhesive properties, which make it an attractive material for detection of lactate. The addition of PVA to PEDOT: PSS enhances the ductility, durability, and flexibility of the resulting films. The effectiveness of this bio-functionalization method was the immobilization of the enzyme lactate oxidase (LOD) on PEDOT: PSS/PVA for development of a lactate sensor.
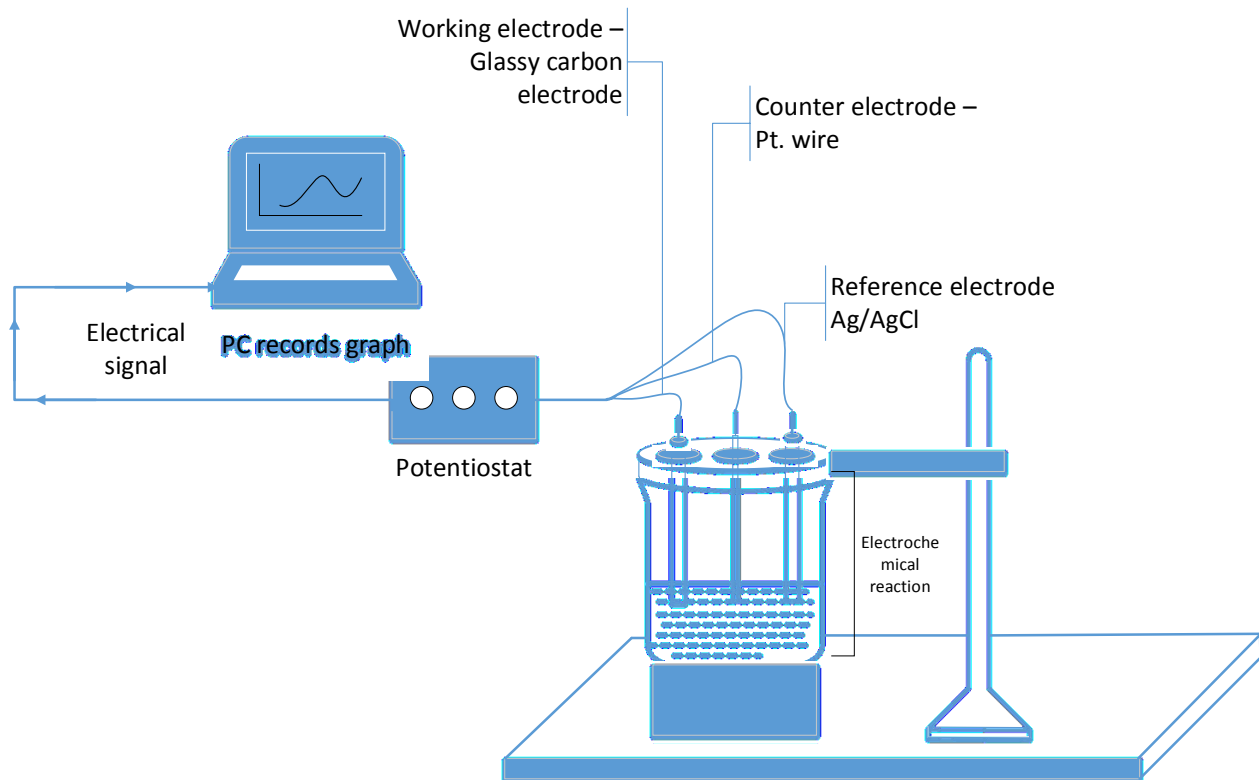
Figure 2 Three-electrode Electrochemical set-up

The sweat lactate profiles were recorded by amperometric methods. Electrochemical characterization was performed at room temperature using a Palm Sens, EmStat3 (potentiostat) (Figure 2). The applied potentials in all measurements were against the screen-printed pseudo Ag/AgCl reference electrode. In the presence of dissolved oxygen, L-lactate oxidase catalyzes the oxidation of L-lactate to pyruvate and forms hydrogen peroxide, which is electrochemically active and can be either reduced or oxidized to give a current proportional to the L-lactate concentration. A highly sensitive and stable lactate sensor based on PEDOT: PSS/PVA has been developed. The unique structure provided a favourable environment to keep the bioactivity of LOD and prevent enzyme molecule leakage. Therefore, the proposed lactate biosensor exhibited good analytical performances including high sensitivity and selectivity with satisfactory stability to amperometric determination of lactate. The results indicated that the proposed PEDOT: PSS/PVA was an attractive matrix for the immobilization of LOD enzymes to fabricate biosensors. The proposed biosensor showed good stability, linearity, sensitivity, and selectivity when it was exposed to lactate test solutions.

Summary

This paper has highlighted the design and formation of amperometric biosensors for the determination of lactate. It also reported the design and the development of compression garment with various panels to provide comfort and placing of sensors within the garment. The biosensor proposed in this research is a non-invasive method to detect blood lactate from body sweat. The findings revealed that PEDOT: PSS/PVA was an effective matrix for immobilising lactate dehydrogenase enzymes for fabricating biosensors. The sensors developed in this study showed good linearity and sensitivity when exposed to lactate test solution. During the next stages the sensors will also be exposed to detect lactate from

artificial and human sweat. The proposed sensors will be evaluated for its efficacy by embedding in garments and conducting trials.

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