

Real-time Continuous Measurement of Lactate through a Minimally-invasive Microneedle Patch: a Phase I Clinical Study

Supplementary appendix I – study design

Supplementary appendix II – microneedle patch fabrication and data processing

Supplementary appendix I

Study design

This is a Phase I first in human clinical study evaluating performance and acceptability of a lactate microneedle patch in healthy volunteers.

Inclusion criteria for the study are:

1. Consenting adults ≥ 18 years old
2. Healthy with no previously diagnosed medical condition from a medical practice or use of any active medications
3. Able to perform moderately intensive exercise on an exercise bicycle without difficulty for at least 30 minutes continuously, and engages in regular aerobic exercise at least twice a week

Exclusion criteria:

1. Active inflammatory skin condition such as eczema or dermatitis
2. Active soft tissue infection or infection at any site
3. Known hypersensitivity to any microneedle component or dressings
4. Presence of any implantable electronic devices such as a pacemaker or stimulators
5. Currently pregnant
6. Active symptoms consistent with, or contact with anyone with COVID-19 in the last 14 days.

If participants responded to the advertisement, they were provided with a participant information leaflet in writing by email, followed by in-person screening and informed consent on the day of the visit by the study physician.

The study protocol was reviewed and approved by London - Bloomsbury Research Ethics Committee (20/LO/0364) and registered on Clinicaltrials.gov on 23rd January 2020 (NCT04238611). The study was sponsored by Imperial College London.

Sample Size

As a first-in-human study a formal sample calculation was not performed – a sample size of 5-15 participants was chosen for pragmatic reasons with early stopping if biosensor performance was replicated reliably over a range of lactate values.

Study Procedures

All participants provided written informed consent and were assigned a study number. Procedures were performed to comply with COVID-19 precautions including the use of appropriate personal protective equipment throughout the study.

Single-use lactate microneedle biosensors were placed on inner forearm skin surface cleaned with 2% chlorhexidine gluconate / 70% isopropyl alcohol and applied using firm thumb pressure for 60 seconds. The biosensor was secured in place with an elasticated strap and had a wired connection to the potentiostat (CHI instruments, USA) with real-time microneedle current displayed on a laptop.

The microneedle biosensor was left in-situ on the forearm 60 minutes for stabilisation. The participant was then asked to cycle on an exercise bicycle (Ergoselect 200, Ergoline Germany) at 60 rpm at power increments of 35 W up to a maximum of 210 W, for a total of 30 minutes according to the protocol. The maximum exercise intensity reached was adjusted throughout the study according to participant ability and preference, but ensuring moderate activity was achieved. Power output values were recorded from the exercise bike. A rest period of 30 minutes with cessation of all exercise followed. Throughout the study period both forearms remained stationary on the handlebars. At the end of the rest period, the biosensor was removed and participants were given a questionnaire and photographs of the forearm obtained.

Blood sampling

Venous lactate was sampled at regular 5-minute intervals over 60 minutes from an indwelling cannula in the contralateral arm to the biosensor using fluoride/oxalate vacutainer blood bottles (BD, USA). Samples were stored at 4°C and processed within 12 hours of collection at a UKAS-accredited laboratory located at North West London Pathology, Hammersmith Hospital UK. All samples were processed on the Architect Ci8200 analyser platform (Abbott, USA) through a colorimetric assay. All blood samples were discarded after 72 hours of analysis in line with local policy.

Microdialysis

Microdialysis is an invasive method to sample and measure substrates in the interstitial fluid. A 63 microdialysis catheter (M Dialysis, Sweden) was inserted into the subcutaneous layer of the forearm using sterile technique. Topical anaesthesia (EMLA 5%) was applied on the skin surface prior to insertion. The inserted catheter was perfused with T1 sterile perfusion liquid (M Dialysis, Sweden) at a rate of 2 µL/minute allowing analytes, including lactate, in the

subcutaneous ISF to diffuse into the catheter for downstream analysis. The catheter outflow was connected to a microfluidic chip housing an online lactate sensor developed in-house at Imperial College London[1], connected to a portable potentiostat, which detected lactate continuously in real time. The microdialysis probe was inserted during 60 minutes of stabilisation, and continued until the end of the study period.

An outline for study procedures is provided in figure 1.

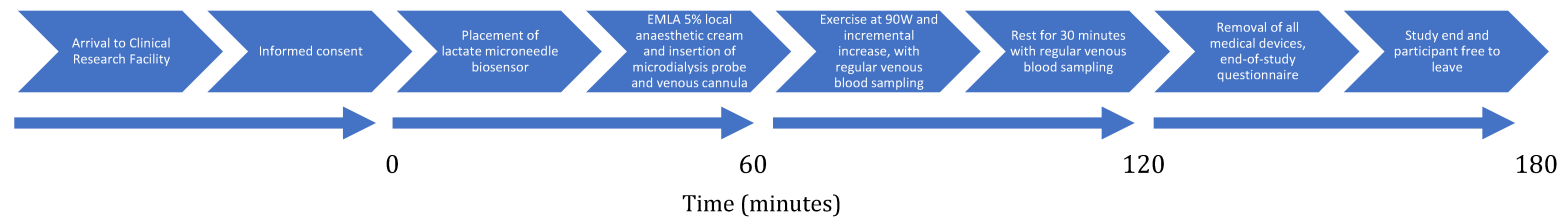


Figure 1 – study outline and timings. The study begins after informed consent with placement of the microneedle biosensor for the 60-minute stabilisation period. During this period venous cannulation and microdialysis procedures are also carried out. The participant is then asked to exercise at 90W with incremental increases in power output for 30 minutes, and asked to rest for another 30 minutes during which venous lactate sampling takes place. Removal of all medical devices and end of study questionnaire takes place after which the study concludes.

Supplementary appendix II

Microneedle patch fabrication

The microneedle platform utilised in the study has been described in full previously [2].

Briefly, the microneedle array (Torr Scientific, UK) is made from a polycarbonate base which measures 2×2 cm and consists of 3 independent working electrode arrays, each consisting of 16 microneedles, and a Ag/AgCl/NaCl reference electrode on a fourth array. A layer of platinum black was applied electrochemically onto the working electrode surfaces followed by deposition of a biocompatible enzyme-hydrogel layer, consisting of lactate oxidase enzyme from *Aerococcus viridans* (Sekisui Diagnostics, Japan), 2% glycerol, 3% w/v bovine serum albumin and 4% polyethylene glycol. Lactate generated in ISF is converted into pyruvate by the embedded enzyme within the hydrogel/skin interface, generating hydrogen peroxide proportionate to lactate concentration.

The resulting hydrogen peroxide change is then amperometrically oxidised leading to a current at the platinum electrode surface measured by a potentiostat applied at 0.7 V at the bedside. An inert membrane consisting of 1-5% Nafion (Sigma Aldrich, USA) was applied onto the electrode surface in multiple layers by nebulisation in order to extend dynamic range of detection. Single-use biosensors were fabricated using a clean process up to a week ahead of the clinical study and kept refrigerated at 4°C until the day of use.

Calibration

Microneedle biosensors were calibrated against L-lactate standards before, and after clinical testing to confirm function. This was done using a tissue phantom technique with insertion of the biosensor into agar gel plates made up with specific lactate concentrations.

For the estimation of ISF lactate concentrations from biosensor we used venous blood levels as a reference. For each result we produced individual calibration plots using corresponding blood lactate concentrations separately for the exercise and rest phase. These derived values were then used for the Bland-Altman analyses (see below).

Microdialysis dialysate results were calibrated to provide concentration values by calibrating the microdialysis sensor against known L-lactate standards to produce a 5-point calibration plot.

Data processing, analysis and statistical methods

A robust LOWESS (locally weighted scatterplot smoothing) filter with a 60-second window was applied to raw microneedle and microdialysis data to remove outliers, motion artefact and attenuate noise.

In order to calibrate individual sensors to blood lactate concentrations, individual standard curves using polynomial functions up to the order 3 using the numpy polyfit function were used. The absolute differences between the values from the standard curve and the actual venous lactate concentrations were then used to produce the Bland-Altman plots. We split the analyses between the exercise (up to 30/35 minutes) and the rest phase (up to 60 minutes) as we assumed that the ISF: blood lactate dynamics to be different between phases. The use of this technique for biosensor calibration is established in other settings such as continuous glucose monitoring.

Venous lactate was assumed to change in a linear fashion based on physiological assumptions with additional data points for venous lactate were interpolated at 1-minute timepoints between the actual blood measurements obtained every 5 minutes. We then plotted microdialysis ISF lactate concentration against the new interpolated venous lactate values as well as against the biosensor current.

In order to analyse the relationship between biosensor current and venous lactate dynamics, and the ability of the biosensor to detect relative change, we normalised all biosensor current and venous lactate data using the MinMaxScaler function from sklearn. This fits individual result values to provide a range of 0 and 1 and allows different participant results to be directly compared and combined.

We used a 5-minute rolling window mean average in order calculate the gradient / rate of change and produced values for every minute of the study for biosensor current and interpolated venous lactate values. The rates of change were then plotted. In order to estimate the lag time between the biosensor and venous lactate we compared rates of change of the biosensor current for each participant with that of venous lactate levels at the point of inflection where lactate/current change changed from positive to negative as a reference, as this point was one which was consistent across individual participants.

All analyses and visualisations were done in Python 3.7 using the *pandas*[3], *numpy*[4], *pyCompare*[5] and *matplotlib*[6] libraries.

References

1. Gowers, S.A.N.; Curto, V.F.; Seneci, C.A.; Wang, C.; Anastasova, S.; Vadgama, P.; Yang, G.-Z.; Boutelle, M.G. 3D Printed Microfluidic Device with Integrated Biosensors for Online Analysis of Subcutaneous Human Microdialysate. *Anal. Chem.* **2015**, *87*, 7763–7770, doi:10.1021/acs.analchem.5b01353.
2. Bollella, P.; Sharma, S.; Cass, A.E.G.; Antiochia, R. Microneedle-Based Biosensor for Minimally-Invasive Lactate Detection. *Biosens Bioelectron* **2019**, *123*, 152–159, doi:10.1016/j.bios.2018.08.010.
3. Reback, J.; McKinney, W.; jbrockmendel; Bossche, J.V. den; Augspurger, T.; Cloud, P.; gfyong; Sinhrks; Klein, A.; Roeschke, M.; et al. *Pandas-Dev/Pandas: Pandas 1.0.3*; Zenodo, 2020;
4. Harris, C.R.; Millman, K.J.; van der Walt, S.J.; Gommers, R.; Virtanen, P.; Cournapeau, D.; Wieser, E.; Taylor, J.; Berg, S.; Smith, N.J.; et al. Array Programming with NumPy. *Nature* **2020**, *585*, 357–362, doi:10.1038/s41586-020-2649-2.
5. jaketmp; Tirrell, L. *Jaketmp/PyCompare*; Zenodo, 2021;
6. Hunter, J.D. Matplotlib: A 2D Graphics Environment. *Computing in Science Engineering* **2007**, *9*, 90–95, doi:10.1109/MCSE.2007.55.