Optical Detection of Lithium Therapeutic Levels in Porcine Interstitial Fluid Collected with Hollow Microneedles

M. Sheikh, M. Qassem, P. A. Kyriacou

***Abstract*—Bipolar disorder (BD), a recurrent chronic disorder characterized by mood fluctuating between episodes of mood elevation and depression, is a leading cause of disability worldwide. Lithium is the most widely used medication for management of BD. However, despite its effectiveness in preventing and reducing mood swings and suicidality, it is a potentially hazardous drug. Lithium has a very narrow therapeutic range (0.4-1.2 mmol/L) with the upper limit being uncomfortably close to toxic levels, hence lithium levels should be monitored regularly. The current techniques of monitoring lithium levels require frequent blood tests and elaborate laboratory methods that cannot be translated into point of care devices for personal monitoring. Dermal interstitial fluid (ISF), an underutilized information-rich biofluid, can be accessed using non-invasive techniques and the lithium concentration in ISF has been found to be proportional to concentration in serum. In the current study a microneedle-based sampling method to extract ISF from porcine skin, as it is similar in anatomy to human skin, was employed. Optical determination of lithium therapeutic concentrations in porcine ISF using a colorimetric method based on the reaction between chromogenic agent Quinizarin and Li^+ ion was then performed. The resulting spectra show spectral variations which are related to lithium concentrations in spiked samples of porcine ISF, hence suggesting the feasibility of utilizing ISF for real-time and minimally-invasive lithium drug monitoring.**

I. Introduction

Bipolar Disorder (BD) is a serious life-long condition, characterised by recurrent episodes of depressed and manic mood states that cause major impairment in the lives of those affected [1]. Lithium remains the "gold standard" for both acute and maintenance treatment of BD. However, the use of lithium must be controlled within a narrow therapeutic window of 0.4-1.2 mmol/L in serum blood as lithium levels above 1.2 mmol/L are considered toxic [2]. The current techniques of blood lithium measurement including flame emission photometry (FEP) and atomic absorption spectroscopy (AAS) are cumbersome and elaborate laboratory methods that cannot be translated into point of care devices for personal monitoring. Dermal interstitial fluid (ISF) is an underutilized information-rich biofluid which can be a proxy for direct blood sampling because of the similar profiles in ISF, serum, and plasma samples. Furthermore, the concentration of lithium in ISF has been found to be proportional to concentrations in serum [3], hence minimally invasive, continuous monitoring of lithium in ISF would offer a more informative measure of this metabolite and will allow bipolar patients to regularly monitor their treatment. We have previously reported the colorimetric determination of lithium levels in artificial ISF based on the reaction between chromogenic agent Quinizarin and Li^+ ion which can be detected using optical spectroscopy in the visible region (400-800 nm) [4]. The present work is aimed at using a similar methodology to achieve determinations of lithium concentration in porcine ISF, as pig stratum corneum is most similar to human stratum corneum and is widely used to study transdermal delivery and skin physiology [5].

Some of the methods of ISF sampling include suction blister [6], which takes approximately an hour to perform and leaves a lasting wound; microdialysis [7], open-flow microperfusion [8], which require minor surgery with significant expertise; and reverse iontophoresis [3] and laser microporation of skin followed by ISF collection under vacuum [9], which need calibration using blood samples and proprietary instrumentation. Several studies have also utilized microneedles, sub-millimeter needle-like structures, which can rupture the first and toughest layer of the skin, the stratum corneum, to access and extract ISF [10][11]. In the current study we have utilized a facile minimally-invasive microneedle-based approach for dermal ISF extraction from porcine skin based on capillary action [12]. Therefore, ISF was extracted from pig skin ex vivo using a microneedle-based extraction tool. The extracted ISF was then spiked with therapeutic concentrations of lithium to achieve optical determination of Li^+ levels in porcine ISF. Optical determination of lithium levels in ISF with high degree of accuracy will allow future developments of a miniaturized and non-invasive device for lithium drug monitoring.

II. Materials and Methods

A. ISF extraction tool fabrication

Stainless steel BD Microfine Pen Needles (4 mm × 32 G) were obtained from Becton Dickinson (New Jersey, United States). Hirschmann 1–5 µl calibrated pipet capillary tubes were purchased from Sigma Aldrich (St. Louis, MO, USA). The needle's small diameter is specifically engineered to limit pain and penetration force and is coated with a silicone lubricant to minimize tissue trauma upon insertion. The microcapillary tube was attached to the pen needle. A clear silicone pipe tubing (0.80mm × 16 mm × 10 mm) was used to cover the microcapillary tube prior to its attachment to the pen needle to cover any gaps between the microneedle and the capillary tube and seal the attachment. A 10 ml BD plastic syringe obtained from Fisher scientific (Waltham, MA, USA)

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was then connected to the other end of the capillary tube and was similarly sealed with a clear silicone pipe tube (Fig. 1). Pulling the plunger of the syringe upon insertion of the microneedle on pig skin surface created a vacuum which facilitated the capillary flow and extraction of ISF.

B. Microneedle-assisted extraction of ISF from pig skin ex vivo

Freshly excised pig abdominal skin was obtained from the local slaughterhouse. The skin was cut using a scalpel into circular samples (4 cm diameter) of 3-5 mm thickness. The skin samples were placed in 50 mm petri dishes (Fisher scientific, Waltham, MA, USA) and then kept inside an environmental chamber (Model: KMF 115, Binder GmbH, Tuttlingen, Germany) at 90% relative humidity (RH) and 25° C for 24 h. This ensured that maximum hydration levels were reached. Following porcine skin sample preparation, the microneedle-based ISF extraction tool was gently pressed into the skin. Upon skin insertion, the syringe plunger was pulled to allow ISF flow into the capillary tube. Moving the ISF extraction tool multiple times allowed for collection of approximately 30 µl of ISF in a 1 h period. The ISF was recovered from the capillary tubes of the microneedle array into a microcentrifuge tube. To elute the sampled ISF, the collected ISF into the microcentrifuge was centrifuged along with 100 µL of deionised water at 1600 rpm for 1 min.

C. Sample preparation

A stock solution of 10 mM Lithium was prepared by dissolving 0.0738 g Li2CO3 (Fisher scientific, Waltham, MA, USA)) in 100 ml dH2O. The Li_2CO_3 solution was then further diluted to make a set of solutions with the following concentrations: 0.4, 0.8, 1.2, 1.6, 2, 2.4, 2.8, 3.2, 3.6, 4 mmol/L. Thereafter, samples of porcine ISF extracted earlier were spiked with lithium by mixing 2.5 µl from each concentration of Li_2CO3 (i.e., .4, 0.8, 1.2, 1.6, 2, 2.4, 2.8, 3.2, 3.6, and 4 mmol/L) with 2.5 µl of porcine ISF. Therefore, 10 samples of 5 µl spiked porcine ISF with lithium concentrations ranging between 0.2-2 mmol/L were achieved.

D. Reference flame photometry measurements

A flame photometer (M410 Sherwood Scientific Ltd, Cambridge, UK) was used to measure the concentration of dissolved lithium in the porcine ISF samples. To perform the reference measurements, the instrument was first left to warm up with the flame alight, and deionised water was aspirated for approximately 30 minutes. A standard solution provided by the manufacturer (containing 1.5 mM of Li+) was then aspirated and the instrument reading was set to 1.5 mmol/L. Measurements of known lithium concentrations from the prepared porcine ISF samples were recorded (Table 1).

E. Optical measurements of lithium in porcine ISF

The following reagents were prepared for colorimetric analysis of lithium using Quinizarin: 0.1 M of NaOH, 0.25 M of Na2CO3, 99.9 % (CH3)2SO and 1 mM of Quinizarin in (CH3)2SO [4]. All chemicals were obtained from Fisher scientific (Waltham, MA, USA). An amount of 5 µl from porcine ISF samples spiked with different concentrations of lithium were pipetted into a test tube. A sample of porcine ISF without lithium was also included as the control. Prepared samples of ISF were then mixed with 10 µL of 0.1 M NaOH, 1 µL of 0.25 M Na2CO3, 4 µL of water, 215 µL of 90 % (CH3)2SO and 5 µL of 1mM of Quinizarin in (CH3)2SO. All samples were kept in a thermostatic bath (Grant InstrumentsTM TC120 Series Heated Circulating Bath) at 25° for 30 mins prior to testing. Data was acquired using a dual beam spectrophotometer (Model: Lambda 1050, PerkinElmer Corp, Waltham, MA) and with the same set up and program settings previously reported [4]. Spectragryph optical spectroscopy software was used to display the spectra, carry out arithmetic and derivative processes, and export data as excel files to generate line graphs and perform regression analysis using IBM SPSS Statistics.

III. Results and Discussion

A. Porcine ISF can be sampled using the fabricated microneedle-based extraction tool

The microneedle-based ISF extraction tool was fabricated using Single BD 32G 4mm Ultra-Fine Pen needles. A glass capillary was then attached to the backing of the microneedle with a 10 ml syringe connected to the capillary tube (Fig. 1A). The ISF extraction tool was fabricated using widely available materials and allowed extraction of an approximately total volume of 30 µl in a 1 h period by moving the extraction tool multiple times along the skin. It is important to note that the combination of the microneedle and the glass capillary without the syringe was not sufficient to allow the flow of the fluid based on capillary action. This was observed as no flow of liquid into the needle-capillary sets occurred when placed into deionized water control droplets. However, attaching a syringe to the other end of the capillary tube and having the syringe plunger pulled, created a vacuum which allowed rapid wicking of the control deionized water droplets as well as extraction of the porcine ISF into the needle-capillary sets. The extracted ISF inside the capillary tube was then collected into a microcentrifuge tube by pushing back the syringe (Fig. 1B and C).

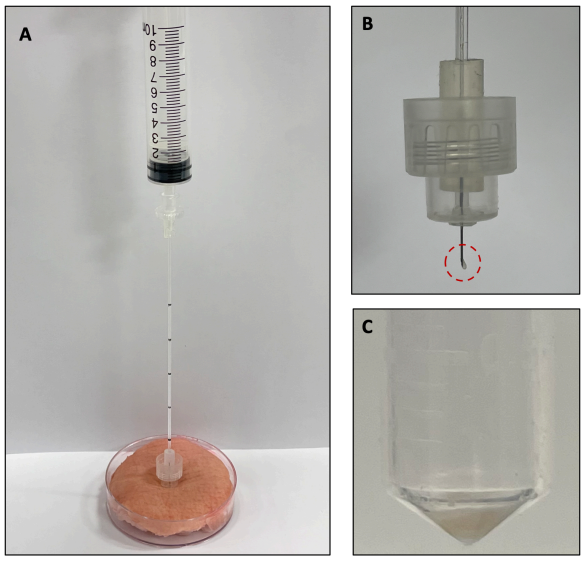

Figure 1. (A) Microneedle-based ISF extraction tool. (B) The sampled porcine ISF in the miconeedle/capillary glass sets before collection into the microcentrifuge for analysis. (C) The sampled porcine ISF (~30 µl).

B. Lithium can be detected in porcine ISF using a colorimetric method based on its reaction with the chromogenic agent Quinizarin

As reported in our previous studies [4][13], the reaction of Li^+ with the chromogenic agent Quinizarin results in the development of a bluish-violet color which allows determination of lithium levels using visible spectroscopy. Therefore, we sought to further investigate this methodology to detect lithium concentrations in drop-volumes of porcine ISF sampled using the fabricated microneedle-based extraction tool. In order to achieve this, the extracted porcine ISF was spiked with therapeutic concentrations of lithium. Therefore, initially samples of porcine ISF, which were prepared to give lithium contents of 0.2-2 mmol/L in order of 0.2 mmol/L, were verified through reference FEP measurements, and are listed in (Table 1). The differences between set and measured Li^+ concentrations are potentially due to minor experimental error and the fact that there are two lithium ions in one molecule of Li_2CO_3. Nonetheless, since FEP is considered the standard in measuring lithium, the remainder of analysis was performed using values extrapolated from the flame photometer measurements.

Table 1. FEP measurements of lithium concentrations in porcine ISF.

Concentration (mM)	FEP Li^+ measurements (mM)
0.2	0.4
0.4	0.8
0.6	1.15
0.8	1.55
1	1.9
1.2	2.25
1.4	2.57
1.6	2.87
1.8	3.2
2	3.5

Thereafter, spectral data of spiked porcine ISF samples in the presence of Quinizarin were acquired using visible spectroscopy and in the spectral region of 400-800 nm. The spectra demonstrated in Fig. 2 represent the peaks of porcine ISF samples spiked with therapeutic concentrations of lithium (0.4-3.5 mmol/L), with two prominent absorption bands around 550-570 nm and 590-610 nm.

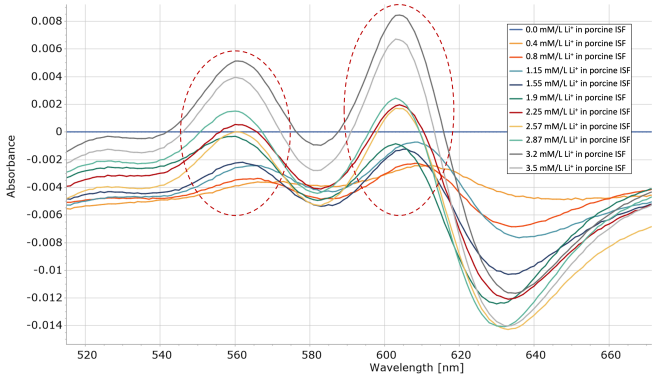

Figure 2. Spectra of therapeutic concentrations of lithium (0.2-2 mM/L) in artificial interstitial fluid, tested using colorimetric method based on the reaction of lithium ion with Quinizarin.

C. The variations in absorption values are proportional to the concentrations of lithium in porcine ISF and can be detected using few selected wavelengths only

In order to separate the peaks of overlapping bands and remove the spectral interferences and baseline effects a second-order derivative was applied which helps to increase the selectivity of the assay. The second derivative spectra of the therapeutic concentrations of lithium in porcine ISF which demonstrate a negative peak for each crest and trough in the spectrum are depicted in Fig. 3. As shown in Fig. 3 the variations in absorption minima between 550-570 nm and 590-610 nm regions correlate with the concentrations of lithium in porcine ISF.

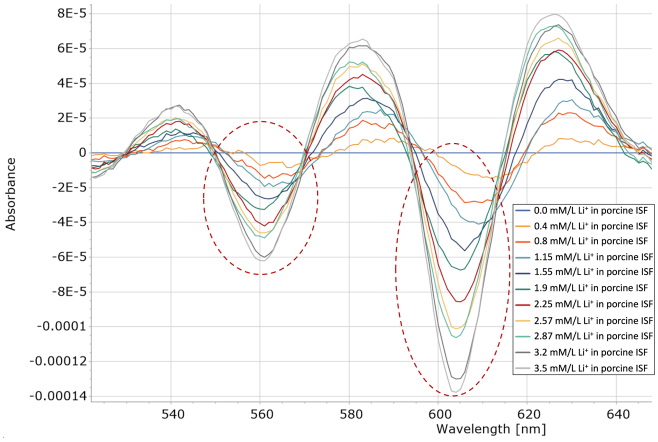

Figure 3. Second derivative spectra of therapeutic concentrations of lithium in porcine interstitial fluid in the range 400-800 nm. Prominent bands found at 610 nm and 560 nm (circled).

Furthermore, to investigate the correlation between the absorption values and the concentrations of lithium, linear regression analysis was performed using two wavelengths from the prominent bands of the second derivative spectra. The regression analysis demonstrated in Fig. 4 suggests that with the increase in concentrations of lithium from 0.4 to 3.5 mmol/L there is a decrease in the absorption values in the second derivative spectra. Moreover, the Coefficient of Determination of 90% achieved from the regression analyses suggests the successful determination of different concentrations of lithium in porcine ISF.

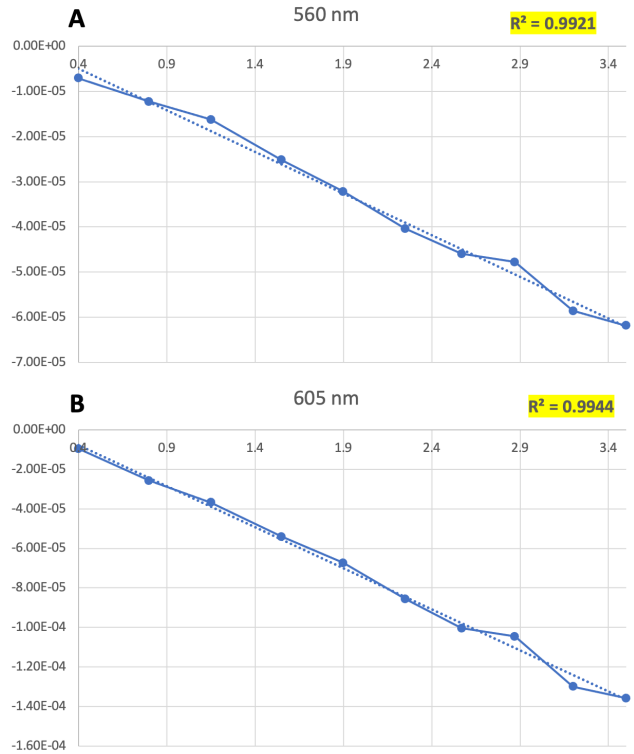

Figure 4. Linear regression analysis of absorption variations of different lithium concentrations. (A) Absorption variations at 560 nm representing the first peak. (B) Absorption variations at 605 nm representing the second peak. (P-value < 0.05)

IV. Conclusions

In conclusion, our study fabricated and utilized a microneedle-based extraction method for ISF fluids in porcine skin. Thereafter, a colorimetric method using chromogenic agent Quinizarin was employed which allowed detection of lithium levels in drop-volumes of porcine ISF. The resulting spectra showed spectral variations which could be related to lithium concentration in spiked samples of porcine ISF. Furthermore, these were used to conduct linear regression analysis which showed a high correlation coefficient, meaning that the absorbance values at the wavelengths of interest were linear and correlated to the lithium concentrations. Therefore, the undertaken studies have demonstrated the feasibility of the proposed colorimetric method for determinations of lithium levels in low volumes of ISF using few selected wavelengths only. This will serve as a basis for our studies in this matrix in human subjects.

Overall, our study supports ISF sampling as a minimally invasive alternative to blood analysis and introduces a new tool for monitoring the dermal interstitium in porcine samples, allowing further characterization of skin physiology and pathophysiology. This extraction tool can be further optimized to achieve minimally invasive ISF extraction in human subjects. Moreover, the results of this study suggest that spectrophotometric measurement of therapeutic levels of lithium in interstitial fluid can provide the monitoring of this medication with an enhanced degree of specificity. Therefore, the results of the current study serve as the foundation for development of a miniaturized and minimally-invasive device for lithium drug monitoring in ISF.

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