Textile-based non-invasive lithium drug monitoring: a proof-of-concept study for wearable sensing

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Abstract: Flexible wearable chemical sensors are emerging tools which target diagnosis and monitoring of medical conditions. One of the potential applications of wearable chemical sensors is therapeutic drug monitoring for drugs that have a narrow therapeutic range such as lithium. We have investigated the possibility of developing a fibre-based device for non-invasive lithium drug monitoring in interstitial fluid. A flexible cotton-based lithium sensor was coupled with a carbon fibre-based reference electrode to obtain a potentiometric device. In vitro reverse iontophoresis experiments were performed to extract Li^+ from under porcine skin by applying a current density of 0.4 mA cm^{-2} via two electrodes. Carbon fibre-based reverse iontophoresis electrodes were fabricated and used instead of a conventional silver wire-based version and comparable results were obtained. The fibre-based Li^+ sensor and reference electrodes were capable of determining the Li^+ concentration in samples collected via reverse iontophoresis and the results compared well to those obtained by ion chromatography. Additionally, biocompatibility of the used materials have been tested. Promising results were obtained which confirm the possibility of monitoring lithium in interstitial fluid using a wearable sensor.

Keywords: Ion-selective electrodes, non-invasive wearable sensors, reverse iontophoresis, lithium drug monitoring, interstitial fluid, biocompatibility.

1. Introduction

Wearable sensors are exciting emerging screening tools which are undergoing active development (Heikenfeld et al. 2018). Many physical wearable sensors have been developed for monitoring of a patient's physiological status, such as temperature, (Webb et al. 2013) heart rate (Zhang et al. 2010) or respiration rate (Reinvuo et al. 2006). While wearable glucose monitors are currently being marketed, few other commercial wearable chemical sensors exist. Using wearable chemical/biochemical sensors at home instead of a traditional clinical setting for screening or follow up would help to reduce the burden on health professional's time such as phlebotomists and nurses. In addition, home and non-invasive monitoring would be friendlier for patients and carers, reducing stress due to blood sampling and time/travel associated with attendance to clinical settings. Such analysis would also be available to a wider patient base including those with poor access to a clinical setting. In addition, non-invasive, home monitoring can facilitate earlier

diagnosis and improved therapeutic monitoring, which may result in better health care, and therapy outcomes thereby improving the quality of life for many people.

Many patients need to monitor their medical condition regularly during treatment. It is estimated that bipolar disorder affects 1 in 100 people (Merikangas and Peters 2010). Lithium remains the most effective long-term therapy for bipolar disorder. Lithium monitoring is essential due to its narrow therapeutic range (0.4 – 1.0 mM) and the toxicity associated with drug-levels above this range (Logan and Main 2013). Bipolar disorder patients need to initially monitor their lithium drug plasma level on a weekly basis then monthly and then every 3 months while treatment with this drug continues (Logan and Main 2013). Lithium is currently quantified in laboratories using atomic spectroscopy and an ion-selective analyser (Christian 1996; Diazyme 2015). A blood sample is taken by a healthcare professional 12 hours after the most recent dose of lithium taken by the patient. The high frequency of invasive blood sampling leads to poor patient compliance which puts the patient at risk of gastrointestinal and nervous system drug toxicity or even death due to the narrow therapeutic range of lithium (Logan and Main 2013). A non-invasive method for measuring drug levels would facilitate monitoring (Djabri et al. 2015) and improve patient outcomes.

Reverse iontophoresis (RI) is one method which has been explored to offer non-invasive therapeutic drug monitoring (Leboulanger et al. 2004c). RI facilitates the extraction of ions and non-charged species from under the skin *via* the application of a small current between two electrodes on the skin (Santi and Guy 1996). Under the effect of this current, ions in the subdermal layer migrate across the skin to the surface where the electrode of opposite charge is located. Extraction of drugs such as sodium valproate (Delgado-Charro and Guy 2003), phenytoin (Leboulanger et al. 2004d), amikacin (Nicoli and Santi 2006) and lithium (Leboulanger et al. 2004a; Leboulanger et al. 2004b; Wascotte et al. 2005) using this non-invasive RI technique has been reported. These studies demonstrated the proof of concept for non-invasive sampling using transdermal iontophoresis, but the collected samples still needed to be sent to a laboratory for analysis. To the best of our knowledge, no study has reported monitoring of therapeutic drugs using a wearable sensor. Fibre-based wearable chemical sensors were previously developed for the determination of pH, K^+ and NH_4^+ (Guinovart et al. 2013) and Na^+ (Parrilla et al. 2016). These sensors required conditioning in a solution of the ion of interest overnight before first use and then for at least 15 mins before each subsequent use. We succeeded in excluding this conditioning step for wearable ion-selective electrodes in our previous work (Sweilam et al. 2018). The exclusion of the pre-conditioning step makes the sensor suited to wearable applications. Another consideration for any wearable sensor is the bio-compatibility of the materials used (Williams 2014).

Here we follow on from our previously reported work and combine a lithium sensor fibre and reference fibre, which can be used without any pre-conditioning step, giving a miniaturised potentiometric cell that can be incorporated in a dermal patch. We report on the performance of an additional set of fibre-based reverse iontophoresis electrodes in extracting lithium ions from under the skin. The extracted lithium ion concentration was subsequently determined using the miniaturised sensor device and compared to analysis using ion chromatography as a reference method. The cytotoxicity of the sensors materials was also investigated as a primary indication of biocompatibility.

2. Methods

2.1. Reagents and solutions

Commercially available carbon fibres (CNF) (93% purity, 6.4 mm length, 0.08 mm diameter) were purchased from Alfa Aesar (Heysham UK). Single-walled carbon nanotubes (SWCNTs >70% carbon purity), were obtained from Nanocyl (Belgium) and used directly without any purification. Lithium ionophore VI (6,6-Dibenzyl-14-crown-4), lithium tetrakis(pentafluorophenyl) borate ethyl etherate (LiTPFPB), trioctylphosphine oxide (TOPO), 2-nitrophenyl octyl ether (NPOE, >99% purity), high molecular weight poly(vinyl chloride) (PVC), Butvar® B-98 , methanol (anhydrous 99.8%), tetrahydrofuran, sodium dodecylbenzenesulfonate (SDBS), Ag wire (99.999 %), platinum wire (99.99+%), AgCl salt (99.999 %) and analytical grade of NaCl, KCl, LiCl salt, Tris base, potassium gluconate (anhydrous), $MgCl_2$ salt and $CaCl_2$ were all purchased from Sigma-Aldrich (UK). Di-sodium hydrogen orthophosphate anhydrous and potassium dihydrogen orthophosphate were purchased from Fisher scientific (UK). Ag/AgCl ink (60/40, C2130809D5) purchased from GWENT part of SunChemical (Torfaen, UK). Cotton thread was purchased from the local shop. Human plasma mixed pool product was purchased from TCS Biosciences Ltd (Buckingham UK). Human keratinocyte cell line (HaCaT) (300493, CLC, Germany), Alamar Blue® (Thermofisher Scientific, UK), Dulbecco's Modified Eagles Medium (DMEM, D6546, Sigma, UK) were used for the biocompatibility assay. Ultrapure water (18.2 MΩ cm) was used throughout this study.

2.2 Fabrication of the lithium sensor and reference electrode. A cotton fibre-based lithium sensor (**CF-Li$^+$**) was fabricated by first dipping 5 mm of a cotton thread in SWCNT ink for 5 cycles. A cycle was composed of dip, dry, wash then dry. Following these 5 cycles, the resulting conductive cotton fibre (dry) was dipped 9 times in lithium membrane solution (Sweilam et al. 2018). The lithium membrane solution was composed of 28 wt % PVC, 68.5 wt% NPOE, 1.5 wt% Li ionophore VI, 0.5 wt % LiTPFPB and 1.5% TOPO. After drying overnight, the resulting sensor could be used directly without preconditioning. A CNF-based reference electrode was fabricated using a 5 mm length of carbon fibre, which was dipped twice in Ag/AgCl ink and cured in an oven at 90 ˚C for 30 min after each dip (**CNF-SI**). The silver-coated carbon fibre was subsequently dipped 5 times in a reference membrane solution (RM) to give the final reference electrode (**CNF-Ref**), which was used directly without any conditioning protocol. The RM solution was composed of 78.1 mg of PVB and 50 mg of NaCl dissolved in 1 ml of anhydrous methanol.

2.3 Potentiometric measurements. An eDAQ potentiostat/galvanostat (ModelEA161) in high z mode and eDAQ e-corder 410 were used to measure the difference in potential between two electrodes. **CF-Li$^+$** and **CNF-Ref** potentials were recorded against a double junction reference electrode (DJRE) Ag/AgCl/KCl (3.0 M KCl) reference electrode part number 6.0726.100, Metrohm, UK, containing aqueous KCl (0.1 M) electrode bridge electrolyte. The total ionic strength and the liquid junction potential was calculated for each calibration point using the Henderson equation. LiCl samples of different volumes were measured using the fabricated lithium sensor **CF-Li$^+$** against the fabricated reference electrode **CNF-Ref**. The Debye-Huckel formalism was used to calculate the activity coefficients.

2.4 Reverse iontophoresis (RI) experiment. A piece of abdominal dermatomed porcine skin was washed, cleaned and prepared for use in iontophoresis experiments. The skin thickness was approximately 750 µm. The skin was stored at -20 ˚C. Franz two-compartment cells were used with 2.0 cm^2 pig skin surface area. The subdermal, anode compartment was filled with 7.4 mL of LiCl (0.2 – 3.0 mM) in PBS (phosphate buffer containing 154 mM NaCl, pH 7.4). Each cathode compartment was filled with 0.3 ml of 30 mM potassium gluconate solution. A constant current of 0.8 mA was applied *via* reverse iontophoresis electrodes (current density = 0.4 mA cm^{-2}) using a KEPCO power supply APH 1000 DM. Carbon-fibre-based reverse iontophoresis electrodes (**CNF-RI**) were fabricated by a single dip in a Ag/AgCl ink to form a uniform coating of Ag/AgCl then

cured in an oven at 90˚C for 30 min. RI experiments started with a 30 min warm-up period, followed by a 2 h reverse iontophoresis lithium extraction step. At the end of the experiment, solutions in the cathode compartments were collected and analysed using the fabricated sensors and ion chromatography. Standard Ag/AgCl wire-based RI electrodes were fabricated for comparison purposes, details of which are provided in the supporting information (Cordery et al. 2019).

2.5 Ion chromatography analysis. Lithium samples were quantified by ion chromatography. The Dionex ICS 5000 instrument was equipped with a Dionex™ IonPac® CS16 (3 x 250 mm) cation-exchange IC column (carboxylate functionalised), CSRS suppressor and Dionex™ EGC III MSA Methane Sulfonic Acid Eluent Generator Cartridge. 30 mM methane sulfonic acid was used with a flow rate of 0.360 ml min^{-1} and sample injection volume of 20 µl. A retention time of 4.5 min was obtained.

2.6 Biocompatibility study. Cell viability studies were performed, in order to investigate the biocompatibility of the **CF-Li$^+$**, **CNF-Ref** and the individual constituents of the **CNF-Ref** (**CNF-SI**, CNF and RM). Specimens of the active 5 mm of electrodes were cut from the rest of cotton/carbon fibres. For sterilisation purposes, the **CF-Li$^+$** sensor, the CNF and the **CNF-SI** were dipped in ethanol for 3h, air-dried and then sterilised for 10 min in a UV/ozone generator (BioForce Nanosciences), while the whole **CNF-Ref** and the RM alone were sterilised with the UV/ozone generator for 15 min, without ethanol sterilization as the RM dissolved in the ethanol solution. The HaCaT was plated in 96-well plates at a plating density of 104 cells/well and allowed to adhere on the surface for 24 h in a humidified atmosphere of 5% CO_2 and 20% O_2 at 37 °C. Afterwards, the sensor specimens were added to each well. Control samples without the sensors were also generated. The cell viability was assessed using Alamar Blue® assay, which incorporated an oxidation-reduction resulting in a color change of the culture according to the cell growth. This assay has negligible toxicity, therefore enables the use of the same HaCaT samples multiple times. Sensor specimens were incubated with the Alamar Blue dye diluted in the cell culture medium according to the manufacturer's instructions for 1.5 h at different culture time points, 1, 3 and 5 d post sensor administration on the cells. The absorbance was measured with a spectrophotometer (Synergy, BioTek, VT, USA) at 570 nm and at 600 nm, as a reference wavelength. Finally, the cell viability was calculated using the dye molar extinction coefficients and appropriate absorbance equations provided by the manufacturer. Optical micrographs of the HaCaT cells in the presence of the sensors on day 1 and day 5 were taken using an inverted phase-contrast microscope with a 10 times dry objective (Axiovert 40, Zeiss, US). Statistical analysis was performed from N = 3 independent experiments with at least triplicate measurements (n = 3) per experiment. Analysis of variance (Two-way ANOVA) was performed using Graph Pad Prism® software with a p-value threshold of 0.05 to evaluate whether there was a statistical difference between the experimental conditions.

3. Results and discussion

3.1 Fabrication of CF-Li$^+$ and CNF-Ref electrodes. A **CF-Li$^+$** thread-based sensor was made as previously reported by coating a cotton thread with SWCNT ink and a lithium-selective membrane (Sweilam et al. 2018). Figure S3 (supporting information) shows an image of the final fibre sensor. A trial to fabricate a pseudo-reference electrode on a commercially available carbon fibre was carried out according to Parrilla et al. (Parrilla, et al. 2016). It involved dipping 5 mm of the carbon fibre in Ag/AgCl ink, followed by oven curing. Carbon fibres dipped twice in Ag/AgCl ink and cured were subsequently coated with a reference membrane comprised of PVB and NaCl; **CNF-Ref** (image available in Figure S3 (supporting information)). The potentiometric response of **CNF-Ref** was measured against the DJRE in 0.1 - 63.0 mM LiCl and a slope of -0.1 mV ± 1.5 mV

dec^{-1} was achieved which is comparable to the reported study (-0.19 mV decade^{-1}). SEM characterisation was carried out to determine the distribution of the coated fibres. A cross section through the **CNF-Ref** electrode showed an inner CNF diameter of 270 µm, a Ag/AgCl film thickness of 460 µm and an outer membrane thickness of 96 µm as shown in Figure S1 (supporting information). Further characterisation of this fibre reference electrode was performed using EDX analysis to investigate if the coated layers overlapped or whether they remained separate from each other. Figure S2 (supporting information) shows EDX elemental mapping in **CNF-Ref**. Well defined, separated layers of CNF, Ag/AgCl and RM are evident.

The **CNF-Ref** electrode could be used directly without pre-conditioning in contrast to the reference electrode developed in Parrilla et al. (Parrilla, et al. 2016) which needed a long overnight conditioning before being used. Conductive cotton-fibres (cotton thread coated with SWCNTs) were tested in place of the inner carbon fibre in **CNF-Ref** and results showed no advantage in using conductive cotton over carbon fibre. For the sake of simplicity, CNF were selected to build reference electrodes on. The **CNF-Ref** electrode took less than 1 min to stabilise after its first contact with solution after which it responded within 20 s. Figure 1 (left) shows the response of four replicate **CF-Li$^+$** sensor electrodes and **CNF-Ref** electrodes against a DJRE with slopes of 59.9 ± 0.6 mV dec^{-1} and -0.1 ± 0.6 mV dec^{-1} respectively. There is clearly a larger variation in the reproducibility of **CNF-Ref** electrodes compared to **CF-Li$^+$** sensor electrodes. Further work is required to improve the response and reproducibility of **CNF-Ref** electrodes.

3.2 Potentiometric measurements on miniaturised fibre-based device (CF-Li$^+$ connected to CNF-Ref). Each of the four **CF-Li$^+$** fibre sensors were randomly connected to a **CNF-Ref** electrode and will be referred to as **Li$^+$-Ref** device. The randomly paired **Li$^+$-Ref** devices were tested in LiCl solutions over a concentration range of 0.1 - 63 mM. Good Nernstian responses were obtained using **Li$^+$-Ref** devices with little variation between replicate measurements as can be seen in Figure 1 (right) with an average slope of 57 ± 1 mV dec^{-1} which maintains a good Nernstian response and compares well to the average response of **CF-Li$^+$** sensor electrodes against DJRE; 59.9 ± 0.6 mV dec^{-1} (Figure 1 left). The % RSD of the standard potential (as indicated by the intercept) for the 4 replicate **Li$^+$-Ref** devices is 3 % which compares well to the % RSD of the intercept of **CF-Li$^+$** sensor electrodes (n = 4) obtained against DJRE (RSD = 1.7 %). The LOD was found to be 10^{-5} M.

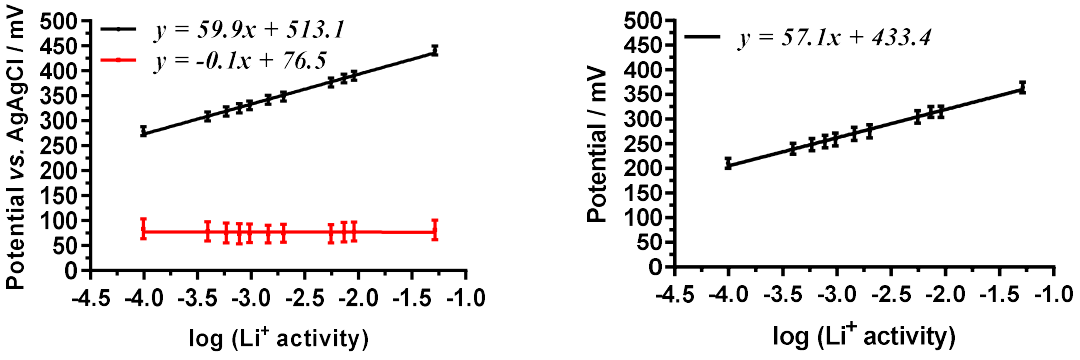

Figure 1: Left: The average potentiometric response of replicate **CF-Li$^+$ & CNF-Ref** electrodes *vs.* Ag/AgCl DJRE. Right: The average potentiometric response of replicate **Li$^+$-Ref** devices. All measurements were performed in 25 ml of aqueous LiCl solution over a concentration range of 0.1 – 63 mM, n = 4.

The slope of the Li^+-Ref device (57 ± 1 mV dec^{-1}) was comparable to the slope obtained for a miniaturized paper-based lithium device reported by Novell et al. (Novell et al., 2014); slope = 57 ± 2 mV dec^{-1}. The main advantage of our device is the possibility to use it without the need for a conditioning protocol while the device reported by Novell et al. needed an overnight conditioning protocol before the first use and a 20 min conditioning before any subsequent use.

Potentiometric measurements up to this point were carried out using a sample volume of 25 ml. Reduction of the sample, to volumes at the microlitre scale, was essential for the intended wearable applications. The **Li^+-Ref** devices were tested in different sample volumes ranging between 50 – 300 µl and the response obtained was compared to those achieved in 25 ml samples. The average slopes calculated for all the devices in different samples volumes ranged between 55.6 – 59.4 mV dec^{-1}. The **Li^+-Ref** devices responded in a similar manner to all sample volumes tested (50, 100, 200 and 300 µl), while the highest precision was achieved with a sample volume of ≥0.3 ml. The results shown in Figure 2 confirm the ability of the **Li^+-Ref** devices to respond to Li^+ concentration changes in a similar manner even with a minimal sample volume. Further optimisation is however required to increase the resiliency of the fibre-based reference electrodes to differences in chloride concentration, which gives rise to changes in the electrode standard potential. The devices therefore require calibration before use to eliminate such differences in standard potential between devices.

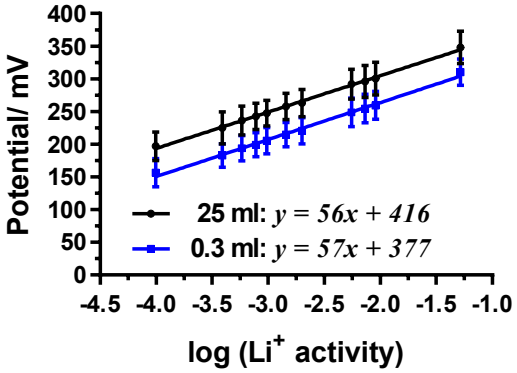

Figure 2: The average potentiometric response of the **Li^+-Ref** device measured in two different sample volumes over a concentration range of 0.1 – 63 mM. The values on the plots are log (Li^+ activity), n = 4 (4 different devices).

The ability of the **Li^+-Ref** devices to respond to continuous changes in Li^+ concentration was investigated. Figure 3 shows a rapid and stable response towards LiCl concentration changes, with 0.3% RSD from one cycle to the next, which is comparable to results obtained in our previous publication when using a DJRE (Sweilam et al. 2018).

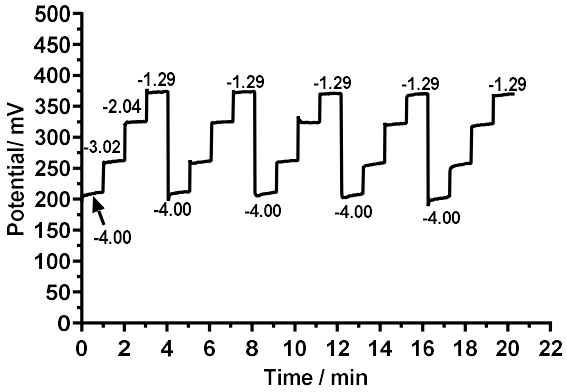

Figure 3: The potentiometric response *vs.* time of a **Li⁺-Ref** device in 0.1 ml of solution when varying the concentrations of LiCl solution (concentration range of 0.1 – 63 mM) and after a sudden reduction in Li^+ concentration. The values on the plots are log (Li^+ activity).

The **Li⁺-Ref** devices were tested in human plasma since it is the most similar biological matrix to interstitial fluid in terms of composition. The human plasma samples were spiked with different concentrations of LiCl and the potentiometric response was measured. Figure 4 shows very promising responses for 5 different **Li⁺-Ref** devices towards Li^+ concentration changes in plasma. The response of the **Li⁺-Ref** device spans the clinically relevant therapeutic range and into the clinically toxic range. The results were comparable to measurements of a **CF-Li⁺** sensor fibre in LiCl spiked plasma samples against a DJRE, Figure 4. The % Recovery of Li^+ concentration using the **Li⁺-Ref** device was 100 (± 3) % compared to 100 (±1) % when using **CF-Li⁺** sensor against a DJRE. These results give a good indication of the capability of the **Li⁺-Ref** device to determine Li^+ concentration in interstitial fluid.

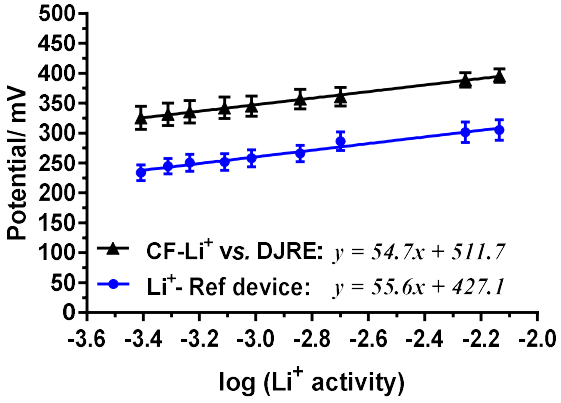

Figure 4: The response of 5 different **Li⁺-Ref** devices measuring Li^+ concentration change in 0.1 ml spiked plasma compared to the response of 3 replicates of **CF-Li⁺** sensors against DJRE.

3.3 RI experiments. Leboulanger *et al.* (Leboulanger et al. 2004a; Leboulanger et al. 2004b; Wascotte et al. 2005) studied the extraction of Li^+ from interstitial fluid *via* RI. Their study was carried out using silver wire-based RI electrodes and 1.5 ml of collecting solution in both the cathode and anode compartments. Changes to this experimental set-up are required for wearable applications. Two main objectives will be discussed here. First, the selection of the collecting

solution electrolyte and the reduction of the collecting solution volume. Second, the replacement of silver wire RI electrodes with flexible carbon fibre-based RI electrodes

In the lithium extraction experiment, two-compartment Franz diffusion cells were used. The anode compartment acted as the subdermal lithium reservoir while the upper cathode compartment was filled with collecting electrolyte. 1.0 ml of KCl, potassium gluconate or Tris buffer were tested as collecting electrolyte in the upper cathode compartment to measure their effect on the extraction rate. 0.1 ml aliquots of this 1.0 ml solution from each cathode compartment (containing KCl, potassium gluconate or Tris buffer) were analysed using the **Li$^+$-Ref** device and potentiometry. In addition, 20 μl of the collected sample was analysed using ion-chromatography and the results were compared to those obtained from the **Li$^+$-Ref** device. Figure 5 shows that the maximum extraction rate was obtained using a potassium gluconate electrolyte which may be due to the difference in size and mobility of Cl^- and gluconate anions (gluconate$^-$ and Cl^- mobility = 2.48 and 8.06×10^{-4} cm^2 s^{-1} V^{-1} (Mudry et al. 2006)). This is consistent with earlier work on iontophoretic transport numbers (Mudry et al. 2006). A paired t-test revealed no significant difference between results obtained by the **Li$^+$-Ref** device and ion-chromatography which indicates that the **Li$^+$-Ref** device is a potential point-of-care alternative to ion-chromatography.

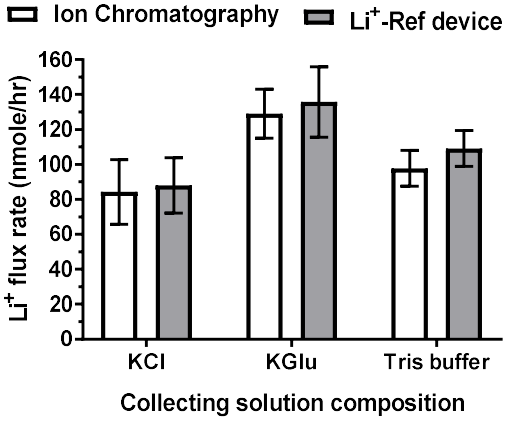

Figure 5: Data of 3 groups of triplicate Franz diffusion cells using 30 mM of different collecting solutions (KCl, potassium gluconate (KGlu) and Tris buffer).

3.4 Replacement of rigid silver wire with flexible CNF-RI electrode. Frequently, a silver wire (~6 cm length, 0.5 mm diameter) is used as RI electrode as described in the methodology. As the aim was to fabricate a wearable lithium extraction and sensing system, a silver wire was considered inappropriate due to its rigidity and overall dimensions. A flexible fibre-based RI electrode was developed on commercially available carbon fibre using a one-step procedure of dipping the carbon fibre in Ag/AgCl ink followed by curing (Figure S3). To investigate the applicability of using **CNF-RI** electrodes in RI experiments, two sets of triplicate Franz diffusion cells were filled with the same subdermal solution concentration (7.4 ml of PBS spiked with 1.5 mM LiCl) and the same collecting cathode solution (1.0 ml of 30 mM potassium gluconate). Silver-based RI electrodes were used in one group of the triplicate Franz diffusion cells while in the other group the **CNF-RI** electrodes were used. Figure 6 shows that good agreement in Li^+ extraction was achieved using the **CNF-RI** electrode in RI experiments compared to the traditional silver wire. The samples collected from the upper cathode compartments were analysed using both the **Li$^+$-Ref** device and ion chromatography and the results obtained were compared to each other. A paired student's t-test revealed no significant difference between the lithium extraction fluxes attained with the two

methods. The results in Figure 6 also show good agreement between measurements made by the **Li$^+$-Ref** device when compared to results obtained by ion chromatography.

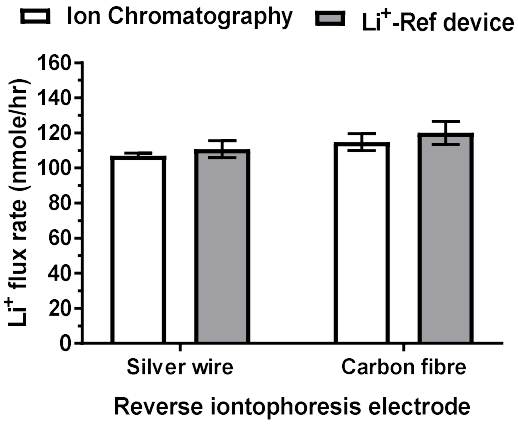

Figure 6: The Li$^+$ flux rate of 2 groups of triplicate Franz cells; 1st group used silver wire-based RI electrodes and 2nd group used flexible **CNF-RI** electrodes.

Figure 6 shows that both the silver wire RI and **CNF-RI** electrodes were capable of extracting the same amount of Li$^+$ from under the porcine skin and confirm that the replacement of the silver wire RI electrodes with carbon fibre-based equivalents did not lead to any significant difference in terms of extraction rate. In addition to the flexibility offered by the **CNF-RI** electrode, the straight forward fabrication methodology made it more favourable to use than the silver-based RI electrode.

The **Li$^+$-Ref** device has a linear response between 0.1 – 63 mM and was capable of determining lithium ion concentration changes in sample volumes ranging from 0.1 – 25 ml. An optimum sample volume was selected to enable both the reverse iontophoresis extraction of lithium ions and the quantification of lithium ions using the **Li$^+$-Ref** device. For the reverse iontophoresis experiment, a current density of 0.4 mA cm^{-2} was applied to 2 cm^2 of porcine skin. A sufficient volume to cover a 2 cm^2 area of skin while maintaining no contact between the RI electrodes and the skin was sought. To allow potentiometric measurements, a minimum sample volume was required to meet the quantification limit of the **Li$^+$-Ref** device. It was theoretically calculated that to work within the linear range of the potentiometric cell a volume of less than 0.5 ml was required. It was found that the minimum sample volume that could cover the skin and provide no contact between the RI electrodes and the skin was 0.3 ml.

3.5 The relationship between subdermal concentrations of Li$^+$ and the extracted concentration. After optimising the collecting solution, the CNF-based RI and the sample volume, *in vitro* investigations into the effect of subdermal LiCl concentration changes were carried out. Different LiCl concentrations (0.2 – 3.0 mM) in PBS acted as subdermal anode solutions. The cathode compartment was filled with 0.3 ml of potassium gluconate. The use of 0.3 ml of potassium gluconate as a collecting solution enabled concentrated samples to be collected at the end of the RI experiment, which were within the linear dynamic range of the **Li$^+$-Ref** device. Figure 7 shows the linear relationship between the subdermal lithium ion concentration and the extracted lithium ion concentration as measured by the **Li$^+$-Ref** device. The results show a good correlation between different subdermal concentrations and the Li$^+$ extracted (*via* RI) and measured using the **Li$^+$-Ref** device. The **Li$^+$-Ref** device shows good linearity above 0.8 mM Li$^+$ and further investigation of the

non-linearity below 0.6 mM Li^+ is required. The usual therapeutic range for lithium blood concentration is between 0.6 – 0.8 mM, with blood tests advised every three months during therapy. Monthly monitoring is recommended for levels above 0.8 mM (Haste 2017). The results from 5 replicate experiments (covering the range of 0.2 – 3.0 mM) show that the **Li^+-Ref** device responses for 0.8 and 1.0 mM Li^+ are significantly different from each other. These initial results show that the **Li^+-Ref** device could act as a warning system that Li^+ levels over the usual therapeutic range had been reached. This initial investigation shows the considerable promise of the **Li^+-Ref** device for efficient management of therapeutic lithium. The electrodes developed here could be incorporated within a skin patch, with connections available to a miniature potentiostat that can deliver wireless electrochemical readouts with smartphones (Kumar Vashist et al. 2015). This could facilitate personalized monitoring of lithium drug levels for patients. Further optimisation of the linear dynamic range of the device may allow better sensitivity within the therapeutic range which will enable more accurate and precise measurements of Li^+ concentration levels.

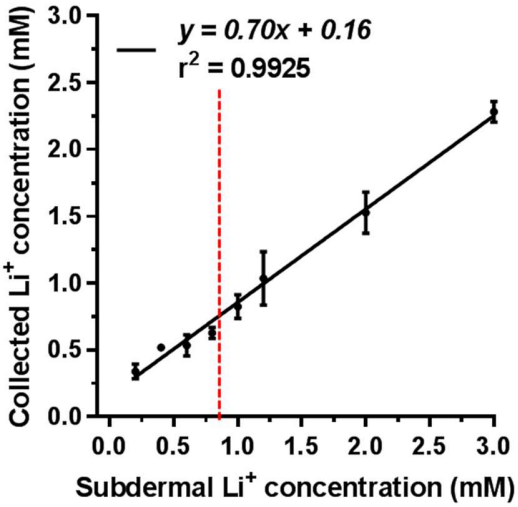

Figure 7: Calibration of reverse iontophoresis extraction. Samples analysed using the **Li^+-Ref** device. The dashed line represents the concentration boundary where three-month testing (LHS) is advised to change to monthly testing (RHS). n = 3 – 5.

3.6 Biocompatibility of the fabricated fibre-based potentiometric cell. To assess the biocompatibility of **CF-Li^+** and **CNF-Ref** fibre electrodes, HaCaT cells were cultured in the presence of both the **CF-Li^+** & **CNF-Ref** fibre electrodes. As can be seen in Figure 8, the cell viability was not significantly affected by the **CF-Li^+** sensor presence (compared to the control) for all the culture duration; therefore this sensor appears to be non-toxic for the cells. However, there was a significant decrease in cell growth (53%) for the cultures including **CNF-Ref** fibre electrodes. This agrees with the visual observations presented in Figure S4, which indicate the undisturbed cell growth for the lithium sensor samples and the reduced number of viable keratinocytes due to the presence of the **CNF-Ref** electrode. In order to further identify the reference sensor's component that triggered the toxicity, HaCaT cultures including the electrodes constituents (**CNF**, **CNF-SI and RM**) were generated. Figure 8 indicates that the **CF and RM** are not toxic, while the **CNF-SI** appears to reduce cell viability. It was observed that the coating of the **CNF-SI** with **RM** decreased the cell toxicity that occurred due to the presence of the silver ink. Many commercially available healthcare electrodes safely use silver chloride, therefore the toxicity observed may be

due to the incomplete evaporation of methyl carbitol and butyl glycol acetate in the silver chloride ink during oven curing. Future investigation of different Ag/AgCl ink is required.

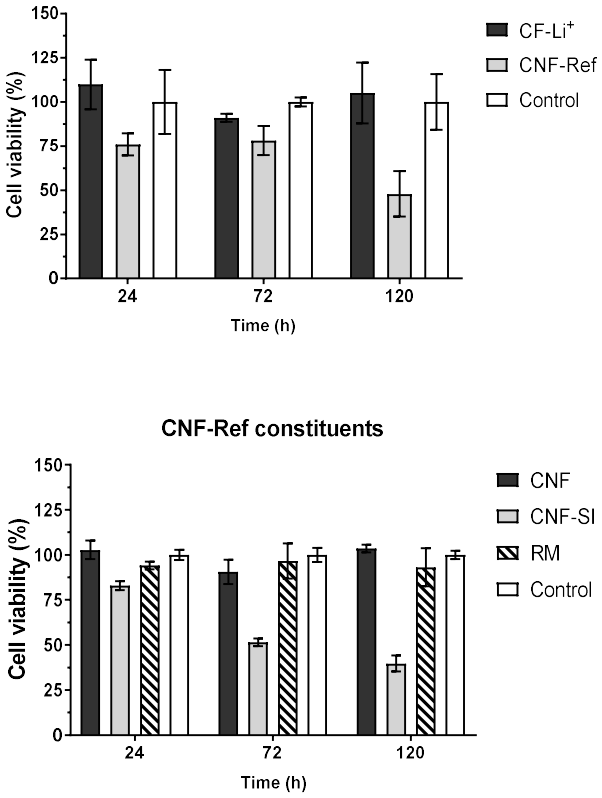

Figure 8: Top: Cell viability of HaCaT cells growing with the lithium sensor, the reference sensor and control samples 24, 72 and 120 h post sensor administration (N = 3, 3 independent experiment with 3 replicate each). Bottom: Cell viability of HaCaT cells growing with the carbon fibre, the carbon fibre coated with silver ink and control samples. n = 3.

4. Conclusions

This proof-of-concept study highlights the promise of a miniaturised, flexible fibre-based potentiometric cell (**Li^+-Ref** device) for non-invasive therapeutic Li^+ monitoring. The potentiometric cells were able to determine Li^+ concentration in plasma as a complex biological fluid. The ability of the fibre-based sensors to determine Li^+ concentration change in simulated interstitial fluid *in vitro* was confirmed. The extraction of Li^+ using flexible fibre-based RI electrodes was carried out. The biocompatibility of **CF-Li^+** sensor was confirmed while the silver ink used in the **CNF-Ref** sensor showed cytotoxicity. The possibility of fabricating reference electrodes on carbon fibre which can be used directly was examined and the biocompatibility of the **CNF** and the **RM** is a good sign for the possible usage of these sensors as a wearable sensor. While challenges remain with highly sensitive detection in the extremely narrow therapeutic range of this drug, this preliminary proof-

of-concept demonstration indicates the potential of fibre-based wearable devices for managing Li^+ therapy.

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