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Hydrogel-forming microneedle arrays: Potential for use in minimally-invasive lithium monitoring

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Abstract
We describe, for the first time, hydrogel-forming microneedle (s) (MN) arrays for minimally-invasive extraction and quantification of lithium in vitro and in vivo. MN arrays, prepared from aqueous blends of hydrolysed poly(methyl-vinylether-co-maleic anhydride) and crosslinked by poly(ethyleneglycol), imbibed interstitial fluid (ISF) upon skin insertion. Such MN were always removed intact. In vitro, mean detected lithium concentrations showed no significant difference following 30 min MN application to excised neonatal porcine skin for lithium citrate concentrations of 0.9 and 2 mmol/l. However, after 1 h application, the mean lithium concentrations extracted were significantly different, being appropriately concentration-dependent. In vivo, rats were orally dosed with lithium citrate equivalent to 15 mg/kg and 30 mg/kg lithium carbonate, respectively. MN arrays were applied 1 h after dosing and removed 1 h later. The two groups, having received different doses, showed no significant difference between lithium concentrations in serum or MN. However, the higher dosed rats demonstrated a lithium concentration extracted from MN arrays equivalent to a mean increase of 22.5% compared to rats which received the lower dose. Hydrogel-forming MN clearly have potential as a minimally-invasive tool for lithium monitoring in outpatient settings. We will now focus on correlation between serum and MN lithium concentrations.

Keywords:
Microneedles
Lithium
Therapeutic drug monitoring

1. Introduction

Despite the introduction of a wide range of mood stabilizing agents, lithium is still considered the ‘gold standard’ treatment for bipolar (BP) disorder [1,2]. As a pharmacological agent, lithium is a cornerstone of neuropsychopharmacology, primarily used to treat BP affective disorder, where it can improve both manic and depressive symptoms [3]. In addition, it also has many off-label uses, including treatment of alcoholism, hyperthyroidism, personality disorders, traumatic brain injury, tardive dyskinesia and postpartum affective psychosis [4]. Lithium, like all alkali metals, is highly reactive [5]. Therefore, it readily forms, and can be administered as, salts such as citrate, sulphate, chloride and, most commonly, carbonate. These salts are available in several different dosage forms, such as syrup and both conventional and sustained release tablets and capsules [4,6].

Early studies reported the effects of lithium on many neurotransmitter and neuromodulatory systems such as the cholinergic, monoaminergic and gamma-aminobutyric acid (GABA) systems [7]. Many theories speculate on the mechanism of lithium’s mood stabilizing action. The initial accepted mechanism of action (MOA) of lithium in BP disorder suggested that lithium interfered with neuronal sodium–potassium electrogenic pumps and, in doing so, altered synaptic transmission [6,7]. However, recent evidence has indicated that lithium may have several MOA, including a direct effect on glutamatergic neural transmission, notably via neuronal excitability at hippocampal cornu ammonis (CA) 1 synapses, thereby exciting the excitatory postsynaptic potentials [7].
Following oral administration, lithium is rapidly absorbed from the gastrointestinal tract (GIT) with a peak plasma concentration ($C_{p,max}$) achieved after 1–5 h, and a half-life ($t_{1/2}$) of approximately 22 h [8]. In addition, lithium does not bind to plasma proteins, has a large volume of distribution (Vd) and can accumulate in many tissues [9]. Depending on its formulation, it has an 80–100% oral bioavailability and steady state concentration ($C_{p,ss}$) in blood is typically achieved within 4–5 days of starting treatment [8–10]. More than 95% of lithium is excreted unchanged through the kidneys. Over 80% of filtered lithium is reabsorbed in the proximal tubules and at $C_{p,ss}$, lithium clearance is directly proportional to the glomerular filtration rate (GFR) [9–11]. The clinically effective plasma concentration of lithium in humans typically ranges from 0.4 to 1.2 mmol/l. This narrow therapeutic window can predispose patients to lithium toxicity with even minor changes in health status or co-prescribed medications. As such, continued lithium therapy requires regular therapeutic drug monitoring (TDM) to ensure optimum clinical benefits without the risk of toxicity [6]. Lithium toxicity in humans is a life threatening condition and typically occurs when serum concentrations exceed 1.5 mmol/l. Adverse effects include muscle weakness, coarse tremor, slurred speech, seizures and irreversible renal damage. Adverse effects of lithium on the kidney and thyroid gland have also been observed during long term use, even when a serum concentration is maintained within the recommended therapeutic window [12]. Moreover, the combination of a reduced Vd and reduced renal clearance of lithium in the elderly frequently leads to a lower tolerability with increasing age and, therefore, neurotoxicity may occur in the elderly at concentrations considered therapeutic in healthy adult populations [13]. Therefore, accurate, precise and regular TDM of lithium is crucial to ensure safe and beneficial treatment of patients. Typical regimes suggest that, upon commencement of lithium therapy, TDM should be performed on a weekly basis. However, once the patient is established on their required therapeutic dose, TDM is typically only performed monthly [14] (see Figs. 1 and 2).

Clinical laboratories employ several methods for the measurement of lithium in serum. These include flame atomic absorption spectrometry (FAAS), flame emission spectrometry (FES), inductively-coupled plasma mass spectrometry (MS) and ion-selective electrodes (ISE) [15,16]. Colorimetric, photometric and enzymatic methods have also been used for TDM purposes [16]. Of these methods, FAAS is the most commonly used technique, due to its sensitivity and simplicity [15–17]. Despite the valuable clinical data provided by TDM, its practical application in clinical settings can be greatly limited by the high cost and time-consuming nature of analytical procedures, as TDM requires repetitive withdrawal of blood samples by nursing or medically-trained personnel using hypodermic needles [18]. Sampling is, therefore, invasive and often painful, especially in patients with difficult venous access [19,20]. In addition to the need for trained personnel, the use of conventional needles is often associated with a risk of infection or transmission of blood-borne diseases, due to accidental needle-stick injuries and/or improper needle disposal [20]. Notably, previous attempts to monitor lithium using either saliva or urine, as alternatives to TDM using blood, have not proven to be successful [20,21]. Therefore, developing a minimally-invasive method would be a major advance in lithium TDM, allowing non-medically trained personnel, or the patient themselves, to monitor their lithium levels, thus ensuring patients are receiving the optimum dose.

ISF has previously been proposed as a viable alternative to plasma or serum as a source for TDM [22]. The composition of

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**Fig. 1.** Diagrammatic representation of steps involved in the preparation of polymeric MN. (A) Polymer matrix was transferred to the silicone mould and (B) centrifuged at 3500 rpm for 15 min. Upon drying for 48 h (C) and heating for 24 h at 80 °C (D) to induce crosslinking via esterification, the silicone mould was carefully peeled away from the polymeric MN array (E) and side walls removed using a hot scalpel blade (F).
ISF closely resembles that of plasma, but without the plasma proteins. Sampling ISF is a potentially non-invasive and painless procedure. In addition, sample preparation and analysis of ISF can be simpler than those of blood, as it contains smaller quantities of cells and proteins [22]. Reverse iontophoresis (RI) has previously been proposed as a viable non-invasive sampling method for lithium TDM, using ISF as an alternative TDM sampling reservoir [19]. However, RI has several problematic issues. These include the need for relatively complicated technology, a ‘warm-up’ time of 2–3 h and the need for preliminary calibration [19,23,24]. These issues present obvious practical problems which could potentially preclude their use by non-trained personnel. Furthermore, in developing countries, where access to the appropriate technology may be limited, the cost of suitable RI equipment may be prohibitive.

MN arrays have recently been proposed by our Group as an alternative approach for sampling ISF [20]. MN arrays are composed of tiny projections (50–900 μm in height) attached to a base support, as previously described [25]. Upon insertion into the skin, MN bypass the stratum corneum, the skin’s outermost barrier layer, and extract ISF without causing pain or bleeding [20,23]. Although MN arrays have been fabricated from a range of materials, including metal, silicon and biodegradable polymers [26] our Group has focused on MN prepared from hydrogel-forming polymers [27]. Such systems consist of a cross-linked polymer network that, upon contact with an aqueous solution, swell to several times their original volume whilst remaining insoluble, therefore maintaining their structural integrity [28]. In their dry state, hydrogel-forming MN possess sufficient mechanical strength to pierce the stratum corneum. However, once in situ they rapidly uptake ISF and, in doing so, can obtain a sample of the analyte of interest. The MN can then be removed intact, allowing subsequent extraction and analysis of the analyte of interest.

Here we describe, for the first time, the use of our hydrogel-forming MN to detect lithium via ISF in vivo. This study aims to evaluate the potential of hydrogel-forming MN as a minimally-invasive tool for in vivo transdermal lithium monitoring.

2. Material and methods

All animal experiments throughout this study were approved by the Ethics Committee of the QUB Biological Services Unit and conducted according to the policy of the European Laboratory Animal Science Associations (FELASA) and The European Convention for the protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, with implementation of the principle of the 3Rs (replacement, reduction, refinement). Any animals with 20% weight loss during the study were to be removed for euthanasia, but this did not occur. No skin reactions to MN occurred either. At the end of the experiment, euthanasia was by carbon dioxide.

2.1. Chemicals

Poly(methylvinylether-co-maelic anhydride) (PMVE/MA) (Gantrez® AN-139) was a gift from Ashland (Kiddermister, UK). Poly(ethylene glycol) (PEG) 10,000 daltons and lithium citrate hydrate were obtained from Sigma–Aldrich, Poole, Dorset, UK. Isoflurane (Isoflo®) was obtained from Abbott Laboratories, Illinois, USA. Reference-grade lithium analytical solution (1000 mg/l) was obtained from J.T. Baker, Radnor, USA. Syringe filters (0.2 μm cellulose acetate) were obtained from Chromacol, Herts, UK. All other chemicals were of analytical reagent grade. Millipore HPLC-grade water was used throughout the study.

2.2. Preparation of hydrogel-forming MN

Aqueous blends containing hydrolysed PMVE/MA (11.1%) and PEG 10,000 (5.6%) were used to fabricate MN arrays (19 × 19 arrays, 600 μm in height, with a base width of 300 μm and inter-spacing of 50 μm) by using laser-engineered silicone micromould templates [27,29]. MN were crosslinked (esterification reaction) [30] by heating at 80 °C for 24 h and sidewalls removed using a heated blade [27,29]. Formulation and mechanical properties of such hydrogel-forming MN have previously been reported [31].

2.3. Mechanical analysis

A TA.XT-plus Texture Analyser (Stable Micsystem, Haslemere, UK) was employed in compression mode, as previously described [31], to investigate the application of varying axial compression loads on the fabricated hydrogel-forming MN arrays. MN were examined under a light microscope (GXMGE-5 digital microscope, Laboratory Analysis Ltd., Devon, UK) before and after application of the compression load to assess the percentage height reduction. Compression forces of 0.18 and 0.7 Newtons (N) per needle were applied, values which are approximately double and tenfold the average manual application force of 30 N (0.083 N/needle), as previously reported [32].

2.4. Optical coherence tomography

Optical coherence tomography (OCT) was used to evaluate the MN penetration characteristics into full thickness neonatal porcine skin (1.0 mm), a good model for human skin [33], obtained from still-born piglets, in vitro. Hydrogel-forming MN arrays were inserted into porcine skin using manual force (thrust pressure) for 60 s, after which adhesive tape was applied to keep them in place. OCT imaging was used to visualize in situ MN penetration as previously described [34]. Images obtained were analysed using Image J® software (National Institutes of Health, Bethesda, MD, USA). Depth and the pore diameter created by MN arrays were measured using Ability Photopaint® Version 4.1.4 (Ability Plus Software Ltd., Crawley, UK) which added additional colour, thereby enhancing the contrast between the skin layer and MN arrays.

2.5. In skin-swelling study

The in-skin swelling characteristics of hydrogel-forming MN arrays were investigated using full-thickness neonatal porcine skin. Prior to testing, the height and base width of 10 MN arrays were measured using a digital light microscope (GXMGE-5 digital microscope, Laboratory Analysis Ltd., Devon, UK). Skin samples were gently shaved and equilibrated in phosphate buffered saline (PBS, pH 7.4) for 15 min. A circular specimen of skin was then dried and mounted on a modified Franz diffusion cell (15 mm orifice diameter, synchronous stirring at 600 rpm and thermostated at 37 ± 1 °C, Crown Glass Co. Inc., Sommerville, NJ, USA) using cyanoacrylate adhesive (Loctite Ltd., Dublin, Ireland), with the stratum corneum accessed as previously described [34]. MN were kept in the skin for 1 h before careful removal and visualization using digital microscopy, which measured both height and width of MN tested. Skin barrier integrity was confirmed as maintained, post-shaving, by using trans-epidermal water loss (TEWL) measurements from a Delfin Vapometer (Delfin Technologies, Surrey, UK).

2.6. Preliminary drug uptake and ability to release study

Preliminary investigations into drug uptake and release were carried out by swelling hydrogel-forming MN arrays for 30 min
in weighing boats containing 20 ml of lithium citrate in PBS solution. Concentrations equivalent to 0.9 and 2 mmol/l lithium carbonate were chosen to replicate therapeutic and toxic concentrations in humans, respectively [35]. After 30 min, MN arrays were removed from the weighing boats, surface PBS removed using filter paper and the MN placed into glass vials. The lithium taken up by the MN was extracted using three different release media: (A) deionized water, (B) 2% v/v hydrochloric acid (HCl, pH = 0.85) and (C) 0.1% w/v potassium chloride (KCl). Three ml of each medium was added separately to each vial containing a swelled MN, and the contents allowed to settle for one min. Each vial was subsequently vortexed for 1 min and all free fluid in each vial collected, filtered and analysed using FAAS at a wavelength of 670.8 nm.

2.7. Lithium recovery from MN

To confirm reproducible recovery of lithium from MN and demonstrate an acceptable extraction efficiency using KCl as an extraction medium, 15 µl of a lithium carbonate standard solution (1000 µg/ml) was spiked onto a MN array. Extraction of spiked lithium from each MN was then performed by placing the spiked MN array into a sterile glass vial containing 2 ml of a 0.1% w/v KCl solution as the extraction medium. As described previously, the glass vial was allowed to settle for one min and vortexed for one min and the remaining free solution was filtered using a 0.2 µm cellulose acetate syringe filter. The filtrate was then aspirated into a FAAS machine for analysis. The percentage recovery (extraction efficiency) was calculated according to Eq. (1):

\[
\text{Extraction efficiency} = \frac{\text{the measured lithium concentration in the extraction medium}}{\text{the expected lithium concentration after full recovery}} \times 100
\]  

2.8. In vitro lithium uptake

In vitro MN-mediated lithium monitoring was conducted across dermatomed neonatal porcine skin (300–400 µm thickness), previously demonstrated as a suitable skin model to predict MN performance in vivo [36]. The neonatal porcine skin was mounted on a modified Franz-cell set-up, as previously described [34] and MN arrays were applied under thumb pressure and held for 60 s. The in vitro uptake study was carried out using Franz-cell receptor chambers thermostated at 37 ± 1 °C, containing two different concentrations of lithium citrate equivalent to 0.9 and 2 mmol/l lithium carbonate. MN arrays were removed at 30 min and 1 h, and lithium was extracted, as outlined above.

2.9. In vivo MN lithium monitoring

Six healthy male SD rats, aged 11–14 weeks and weighing 300–400 g, were divided into two groups (1 and 2), with three rats in each group. Groups 1 and 2 were administered lithium citrate, via oral gavage, equivalent to 15 and 30 mg/kg body weight, respectively. Lithium was dosed in the form of the citrate salt rather than lithium carbonate due to lithium citrate’s higher water solubility at room temperature (74.5 g/100 g) compared to that of lithium carbonate (1.29 g/100 g) [37]. One h after gavage, four MN arrays were applied to the shaved, hairless back of each SD rat and left in place for 1 h. TEWL measurements were again used to confirm maintenance of skin barrier function. An adhesive patch was then applied to further secure the MN arrays, whilst an occlusive dressing was applied to cover the surrounding area to prevent the rats from removing the MN arrays [38]. Following MN removal, blood samples were collected via cardiac puncture.

2.10. Serum preparation method

For assay method development, fresh rat blood was obtained from healthy SD rats. Serum was separated by incubating tubes containing blood at room temperature for one h to allow clotting to occur. The tubes were then centrifuged at 1500 Relative Centrifugal Force (RCF) for 15 min and 200 µl of the supernatant was transferred to disposable glass culture vials. Following this, 200 µl of 20% w/v trichloroacetic acid (TCA) was added to 200 µl serum in the glass vials to precipitate proteins. This was followed by addition of 1600 µl of a 0.1% w/v KCl solution as a diluting medium. The glass tube was then placed on ice for 30 min followed by centrifugation at room temperature for 10 min at 6000 rpm. The supernatant was placed in a fresh glass culture tube and again centrifuged for 5 min at 6000 rpm. The resulting supernatant was then directly aspirated into the FAAS machine for analysis.

2.11. Analytical technique

A flame atomic absorption spectrophotometer (Shimadzu, AA-6300, Tokyo, Japan) was used for determination of lithium ion concentration in all samples. Determination of lithium ions concentration was made at 670.8 nm and a burner height of 7 mm using a pre-mixed air-acetylene flame. The device was operated in the absorption mode, using an SR-lithium hollow cathode lamp (SIM-LabHut Ltd., Gloucestershire, UK) at a current of 8 mA. Least square linear regression and correlation analysis were carried out on the calibration curves obtained to determine equation of the line, coefficient of determination and residual sum of squares (RSS). Standard lithium solutions of 5 µmol/l in KCl (0.1% w/v solution) and 0.1 mmol/l in serum were used to determine the lower limit of quantification (LLoQ). Five replicates were used and precision and accuracy determined for each concentration. Linearity, precision, accuracy and recovery analysis were conducted according to International Conference on Harmonisation (ICH) guidelines [39]. The accuracy and precision of the method were determined from analysis of samples at three concentrations, representing the low, medium and high sections of the standard curves (LQC, MQC and HQC respectively) [39]. Specifically, concentrations of 0.03, 0.07 and 0.15 mmol/l in KCl 0.1% w/v and of 0.7, 1.2 and 1.8 mmol/l in serum were used as LQC, MQC and HQC, respectively.

2.12. Statistical analysis

Statistical comparisons were conducted where appropriate using a t-test or one or two-way analysis of variance (ANOVA). In all analyses, a p value < 0.05 denoted statistical significance. The statistical analysis was performed using Graph Pad® Prism software Version 5.03, CA, USA.

3. Results and discussion

MN technology represents a new and exciting approach for minimally-invasive TDM and diagnosis [26], allowing sampling of
drug molecules from skin ISF without causing pain or bleeding. Our Group has focused on hydrogel-forming MN, which are hard when dry but, upon insertion into skin, imbibe ISF from skin and turn into discrete in situ hydrogel bulbs (Fig. 4b), which can then be removed intact. Upon removal, an offsite analysis is currently used to detect the target analyte [20]. However, future work in MN technology could exploit Internet-based off-site analysis, or potentially incorporate sensors in situ ('lab on a chip' technology) to monitor the drug of interest [40]. In addition, hydrogel-forming MN offer further advantages over hypodermic needles, including elimination of pain, erythema, needle-stick injuries and risk of infections [18,41].

3.1. Mechanical analysis

As shown in Fig. 3, results obtained from the compression tests demonstrate that the reduction in MN height increased with an increase in application force (p < 0.05). The minimum force applied was 0.18 N/needle, equal to 64.98 N/array, greater than double the average achievable manual force and close to the maximum manual force value. The maximum force applied was 0.7 N/needle which equals ten times the average manual force, as described previously [32]. As shown in Fig. 5b and c, despite application of high compressional forces, MN arrays did not break when pressed against the aluminium plate.

3.2. OCT analysis

OCT has the ability to visualize MN penetration into the skin and observe subsequent dissolving or swelling patterns. In addition, previous studies have demonstrated its potential as a tool to study MN penetration depth across the stratum corneum [34]. As shown in Fig. 4a, MN arrays were able to penetrate full thickness neonatal porcine skin upon application, with an insertion depth and pore size diameter of 229.4 ± 17.7 and 195.58 ± 19 μm, respectively. As shown in Fig. 4b, following 1 h insertion, MN arrays had swollen considerably and, in doing so, lost their sharpness, as is apparent in Fig. 4a. Crucially, however, they remained intact, allowing complete removal. This confirms that swelling does not result in ejection of MN from the skin and highlights the safety of our hydrogel-forming MN technology as a tool for TDM without risk of infection caused by accidental needle-stick injuries, an issue of concern highlighted as a drawback for MN prepared from other materials [41].

3.3. In-skin swelling

The swelling of MN arrays following insertion into the skin was investigated in vitro over 1 h. Individual needles on the array exhibited an increase in height and width of 9.2 ± 2.9% and 19.13 ± 2.9%, respectively. Notably, these hydrogel MN arrays imbibed PBS fluid and were swollen, but crucially, remained in situ and again were removed intact.

3.4. Drug uptake and extraction media

As shown in Table 1, extraction of lithium from hydrogel-forming MN bathed in PBS solutions containing different
concentrations (0.9 and 2 mmol/l) of lithium citrate, and subsequently analysed by FAAS, increased with the increased lithium concentration in the bathing solution. This was the case for all extraction media. However, extraction of lithium using HCl 2% v/v (pH = 0.85) was significantly more efficient when compared to deionized water as an extraction medium \((p < 0.05)\). This may potentially be due to the associated increased concentration of protons \((H^+)\) in the acidic HCl solution. As protons possess a higher charge density than lithium ions, and carboxylate ions show greater affinity to such cations, it is possible that protons are substituted for the lithium ions of the carboxylate group in the MN matrix [42]. Similarly, KCl 0.1% w/v solution was studied as a potential medium for lithium extraction. Statistically, there was no difference between the extraction efficiency of HCl 2% v/v solution when compared to 0.1% w/v KCl solution \((p < 0.05)\). Based on these findings, KCl 0.1% solution was chosen as the extraction medium for the remainder of the study due to its lower potential for toxicity and greater safety when handling.

3.5. Validation of analytical method using KCI as extraction media

FAAS was used for quantification of lithium in both serum and KCl solutions. All data were validated according to ICH guidelines [39]. The slope, \(R^2\) and limits of quantification are summarized in Table 2.

The drug uptake and release findings in combination show the KCl solution to be a suitable extraction medium for lithium. Furthermore, the presence of potassium potentially suppresses the ionization of other easily ionized cations, such as sodium, and minimizes chemical interference [43,44]. Chemical interference in transdermal ion analysis has been attributed to small, mobile cations, such as sodium and potassium, which are present in constant concentrations and can be transported across the skin in a similar way to lithium [21] and, therefore, could be simultaneously extracted. Potassium ions in solution do not interfere in the lithium quantification as they were present at a constant concentration in all extractions.

3.6. Lithium recovery from MN

As shown in Table 3, lithium extraction efficiency from spiked MN was 88.68 ± 7.8%.

This percentage recovery indicates that extraction of lithium from hydrogel-forming MN using the method developed is highly efficient. Significantly, the percentage recovery in this study falls within recommended guidelines which accept deviation of ±0.3 mmol/l (or 20%) as acceptable error limits based on United States Food and Drug Administration (FDA) guidelines [45].

3.7. In vitro lithium uptake

As shown in Fig. 6, a significant increase \((p < 0.05)\) in extracted lithium concentration was observed when MN insertion time was increased from 30 min to 1 h. Notably, this effect was observed for both lithium concentrations (0.9 and 2 mmol/l). Although no significant difference \((p > 0.05)\) was observed between extracted lithium values following in vitro MN application after 30 min, a highly significant difference \((p < 0.01)\) was observed in lithium

| Extracting media | Concentration extracted from MN bathed in 0.9 mmol/l solution | Concentration extracted from MN bathed in 2 mmol/l solution |
|------------------|---------------------------------------------------------------|-------------------------------------------------------------|
| Deionized water  | 4.5 ± 2.6                                                     | 318.1 ± 12.7                                               |
| HCl 2% v/v       | 87.7 ± 5.3                                                   | 131.3 ± 12.1                                               |
| KCL 0.1% w/v     | 83.7 ± 5.9                                                   | 123.5 ± 8.3                                                |

| Slope | y-Intercept | \(R^2\) | LoQ (mmole/l) |
|-------|-------------|---------|--------------|
| Serum | 0.0161      | 0.0004  | 0.99         | 0.1          |
| KCl   | 0.1530      | 0.0006  | 0.99         | 0.005        |

Fig. 5. Digital microscope images of hydrogel-forming MN following application of compressional forces (0.18 and 0.7 N/needle) (Means ± SD, n = 3). (a) 19 × 19 MN array before application of compressional force. (b) 19 × 19 MN array after application of compressional force equal to 0.18 N/needle. (c) 19 × 19 MN array after application of compressional force equal to 0.7 N/needle.
concentrations extracted from MN following one h application. Crucially, these results demonstrate that hydrogel-forming MN have the ability to definitively differentiate between therapeutic and toxic ISF lithium concentrations following an application time of only 1 h. This time frame confers a substantial practical advantage when compared with other proposed transdermal TDM methods, such as RI, which may require an application time of up to 5 h [21]. Following application of MN arrays, diffusion along the concentration gradient is the main driving force for solute movement into the MN polymeric matrix. It is, therefore, reasonable to assume that diffusion of lithium into the MN matrix is directly related to its concentration in the Franz cells. In this study, the application time of 1 h appears to reflect the minimum time required for a sufficient volume of fluid to enter the MN array and, subsequently, allow differentiation between a therapeutic and toxic concentration of the analyte of interest. This, therefore, may be the limiting factor in a minimum application time of 1 h using hydrogel-forming MN. In addition, although lithium concentrations from the two Franz-cell chambers were not statistically different following a MN application time of 30 min, they were still quantifiable using FAAS analysis. As such, these findings led us to proceed to the in vivo component of the study.

3.8. In vivo MN lithium monitoring

Due to its narrow therapeutic target range, and its variable serum half-life, which increases with chronic administration [35], regular lithium TDM is required in order to avoid both sub-therapeutic and toxic effects. Toxicity typically occurs when the serum concentration exceeds 1.5 mmol/l for a prolonged period, e.g. several days. As the serum concentration increases, toxic effects include tremor, GIT and respiratory complications, seizures, and finally death [4]. The doses of lithium administered to SD rats in this in vivo study were based on both the paediatric dose (15–60 mg/kg/day, administered in three to four doses) and the adult dose (900–1800 mg/day ≈ 15 mg/kg/day, usually divided in two doses) as indicated for treatment of acute episodes of bipolar mania [4].

Although the kinetic profiles of lithium in humans and rodents differ (Table 4) and identical serum concentration profiles may not be reproducible [46], the study was undertaken as a vital in vivo TDM proof of concept.

In this study, MN arrays were used to capture ISF from six SD rats following administration via oral gavage of lithium carbonate solution. The six rats were divided into two groups and the 1 h application time for MN arrays was chosen based on the findings from the in vitro work previously conducted.

As shown in Fig. 7 the mean serum lithium concentration for Group 1 rats dosed with 15 mg/kg lithium carbonate was 0.1 ± 0.08 mmol/l. In comparison, Group 2 rats dosed with 30 mg/kg lithium carbonate displayed a statistically significant (p < 0.05) difference in serum concentration of 0.24 ± 0.06 mmol/l. Similarly, when lithium levels extracted from MN applied for one hour were analysed, the Group 1 and Group 2 rats displayed concentrations of 4.0 ± 2.5 μmol/l and 4.9 ± 2.2 μmol/l respectively, equivalent to a percentage increase of 22.5%. Although not statistically significant (p > 0.05) these findings suggest hydrogel-forming MN are capable of differentiating between therapeutic and toxic concentrations of lithium in vivo after only one h application time. As transdermal drug extraction and analysis is a multi-factorial procedure, affected by many physicochemical and pharmacokinetic factors, it is our opinion that this variability is an expected challenge of a novel, proof of concept TDM process. However, the results obtained

Table 4
Pharmacokinetic parameters of lithium in humans (Mean ± SD) and rats [46].

| Half-life (h) | Volume of distribution (L/kg) | Clearance (ml/minute) |
|--------------|------------------------------|----------------------|
| Rat          | 6.07                         | 1.13                 | 2.15                 |
| Human        | 28.9 ± 7.9                   | 0.79 ± 0.34          | 24.4 ± 8.0           |

Fig. 6. Concentration of lithium extracted from hydrogel-forming MN after insertion into neonatal porcine skin mounted on modified Franz cells and bathed on the underside by 0.9 and 2 mmol/l lithium citrate in PBS (Means ± SD, n = 4). (* = significant. ** = highly significant.)

Fig. 7. Serum lithium concentration detected in plasma versus levels detected from MN arrays inserted in Sprague–Dawley rats for 1 h following administration via oral gavage of 15 mg/kg lithium carbonate (Group 1) and 30 mg/kg lithium carbonate (Group 2) respectively. (Mean ± SD, n = 3) (* = significant).
demonstrate that MN can capture a quantifiable amount of lithium from ISF following a period of only 1 h application in vivo. Notably, lithium ISF concentration is not instantaneously related to serum concentration [21] and, accordingly, no lithium was detected from MN arrays inserted into the rats’ backs for 1 h prior to dosing. This served as an informative negative control. This strongly suggests that the swelling of MN was due to uptake of ISF containing lithium, rather than any lithium potentially present in rat perspiration. In addition, our polymeric MN systems were removed macroscopically intact after skin insertion, leaving no detectable polymer behind.

Toxic lithium concentration in serum appears one or two days before toxicity symptoms due to a well-established latency effect [6]. Therefore, clinical presentation of lithium toxicity may not be supported by blood level and vice versa [6]. The concept of outpatients using hydrogel-forming MN for lithium TDM would be a significant breakthrough both in routine TDM and in anticipating occurrence of toxic symptoms, facilitating early intervention before serious side effects occur. This could, in turn, save time and costs to the health system, not to mention the obvious patient benefit. As therapeutic lithium levels are based on the serum samples taken in the morning, usually 12 h following the last administered dose [10], patients could apply the MN for one hour in the morning, and then send them to the nominated laboratory for analysis. Lithium from these MN would then be extracted and analysed and the results could potentially be sent to the patients within 24 h, allowing rapid optimization of dose. The current study has the limitations of being carried out in small number of animals. However, as a proof of concept, the obtained animal data are, nonetheless, exiting. Furthermore, the current study was conducted using therapeutic lithium doses, as administration of toxic doses was not ethically acceptable. Nevertheless, the agreement between the extraction of lithium from MN and lithium serum concentration in the two groups of rats suggests real potential for using MN as a therapeutic monitoring tool for lithium.

4. Conclusion

This study demonstrates, for the first time, the potential of hydrogel-forming MN as a means of transdermal lithium TDM using ISF as a sampling reservoir. We have successfully demonstrated hydrogel-forming MN have the potential as a means of minimally-invasive lithium TDM. The findings of this study strongly suggest that hydrogel-forming MN offer an exciting, new means of lithium TDM. Future work will focus on optimization of MN application time, with respect to ensuring precise quantification of lithium concentration, potentially including ‘lab-on-a- chip’ or MN sensor technology to deliver an concentration easily interpreted by both clinicians and patients.

It is expected that hydrogel-forming MN will likely focus on minimally-invasive home-based monitoring, ensuring compliance and preventing toxicity. For home sampling, a short training session may not even be necessary. The patient may learn the technique from an instruction leaflet or an associated website. Regarding sample extraction and analysis, there are currently no portable devices for analysis to be used by a patient. This concept has enormous potential, especially if an analytical device with a display screen or colour change indicator is developed. This would simply provide the clinician and patient with a ‘Yes/No—High/Low’ feedback, allowing them to make simple pre-ordained decisions without involvement of health care professionals. Improved rates of lithium TDM will undoubtedly minimize the chances of adverse events. The existing gap between the standard and current practice of biochemical monitoring of lithium treatment [47] might be replaced with a system such as our hydrogel-forming MN system that can offer frequent, simple and minimally-invasive monitoring in conjunction with fast analysis and feedback. Finally, although this study focused on the quantification of lithium, it could equally be implemented in detection and quantification of other analytes of interest, such as potassium and sodium. These are often deranged in cardiac and renal disorders and as they are also physiological cations, they should be readily detectable using our novel hydrogel-forming MN technology.

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