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Cortisol extraction through human skin by reverse iontophoresis

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ABSTRACT

Continuous monitoring of cortisol at the surface of the skin would advance the diagnosis and treatment of cortisol-related diseases, or of elevated cortisol levels related to stress in otherwise healthy populations. Reliable and accurate detection of cortisol at the skin surface remains a limiting factor in real-time monitoring of cortisol. To address this limitation, cortisol extraction through excised human skin by reverse iontophoresis was studied *in vitro* in side-by-side diffusion cells using a radiolabeled probe. The skin was subjected to four direct current regimens (0, 28, 56, 113 μA cm^{-2}) with the anode in the donor chamber and the cumulative cortisol concentrations recorded in the receiver chamber. The 56 and 113 μA cm^{-2} regimens significantly increased transport of ^{3}H-cortisol through the skin, and current density correlated directly with transcutaneous transport of ^{3}H-cortisol. The threshold of detection of electroosmotic *versus* passive diffusion of cortisol through the skin was between 28 and 56 μA cm^{-2}. The results of this study are significant in examining how lipophilic analytes found in the bloodstream respond to reverse iontophoresis across the skin. In addition, a device integration technique is presented which illustrates how continuous cortisol extraction and sensing could potentially be achieved in a conventional wearable format.

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1. Introduction

Cortisol is a stress hormone that regulates several important physiological processes in the body. Cortisol-related diseases such as congenital adrenal hyperplasia (CAH) and Addison's disease can be diagnosed by a lack of cortisol, whereas an increased level of cortisol is a symptom of Cushing's syndrome [1]. Chronically increased levels of cortisol related to stress can also occur in otherwise healthy populations, incurring depression, heart attack, decreased immune function, and neurological disorders [2]. Cortisol secretion follows a circadian rhythm throughout the day, with levels highest within 30 min of waking and lowest before sleeping [1,3]. Besides this normal rhythm, cortisol secretion is dependent on varying environmental and behavioral triggers [2]. Thus, being able to routinely quantify systemic cortisol has been of growing interest. Furthermore, the development of wearable sensors for continuous monitoring of cortisol levels would allow for real-time monitoring of stress and aid in the management of cortisol-related diseases. Increases in cortisol under stress can be rapid (minutes), and remain elevated while the stressor is present [4]. However, often most important from a health perspective is the return of cortisol to a normal baseline level [5]. Baseline measurements can be made over hours to determine healthy cortisol levels in a particular individual. If post-stress cortisol levels do not return to baseline, then a warning can be issued to alert observers to this response.

Cortisol can be found in a variety of biofluids such as blood, saliva, urine, interstitial fluid, and sweat [1,6]. Various methods have been developed for quantifying cortisol in these matrices for the purpose of point-of-care monitoring, including chromatographic techniques, immunoassays, and immunosensors; an excellent review can be found in Kaushik et al. [1]. However, emerging technologies for the continuous monitoring of cortisol have been hindered by limitations of quantifying this analyte in the biofluids. Many commercially successful and ergonomic technologies for biomonitoring simply interface with skin, which historically has been limited to non-specific optical or electrical measurement techniques [7]. The purpose of this study was to determine if continuous monitoring of systemic cortisol levels would be possible using reverse iontophoresis to extract the analyte through human skin. The skin is negatively charged at physiologic pH, hence it is permselective to cations [8]. "Reverse iontophoresis" is the term commonly used to describe the process in which a small voltage (1–4 V) is applied across the tissue with the anode (or positive potential) beneath the skin surface and the cathode above it, thereby inducing an electric current and an associated electroosmotic flow of water directed from

Abbreviations: SC, stratum corneum.

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the dermis to the skin surface. Uncharged solutes in the water are carried along with the flow [8]. This was the principle behind the GlucoWatch™ topical glucose monitor [9]. This technique could potentially be employed in a minimally invasive wearable cortisol sensor, in which interstitial cortisol levels (rather than interstitial glucose) would be measured.

In order to determine the efficiency of such an approach, this concept was tested through an *in vitro* study in which both passive and electroosmotic transport of a radiolabeled cortisol probe across human skin was measured. Iontophoretic and/or electroosmotic devices employing current densities in the range 100–300 μA cm^{-2} have been approved by the US FDA [9–12]; however, these devices approach the limit of skin tolerance. One is no longer on the market [9] and a second recently had sales suspended due to skin tolerance issues [12]. To minimize skin irritation, we propose that a current density of 50 μA cm^{-2} or lower is a desirable target for frequent iontophoretic treatments. This level coincides with current densities that cause minimal skin resistance changes during studies of excised human skin *in vitro* [13–15]. The focus of the present study was to determine the threshold current density for differentiating electroosmotic cortisol flux from passive cortisol flux, to better understand the prospects for continuous monitoring based on this technology.

2. Materials and methods

2.1. Materials

[1, 2, 6, 7-^{3}H(N)]-Hydrocortisone (92.9 Ci $mmol^{-1}$) in ethanol was purchased from Perkin-Elmer (Waltham, MA). Dulbecco's phosphate-buffered saline (PBS), pH 7.4, was purchased from Sigma-Aldrich (St. Louis, MO). Sodium azide (Thermo Fisher Scientific, Waltham, MA) was added to the buffer at 0.02% to inhibit microbial growth. Human cadaver skin, dermatomed to a thickness of 300–400 μm, was obtained from the New York Firefighters Skin Bank (New York City, NY) and stored at −80 °C until use. A total of 6 skin donors were used in this study.

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2.2. Experimental methods

On the afternoon prior to the start of each experiment, the split-thickness human cadaver skin was removed from the −80 °C, thawed rapidly in room temperature Millipore water (18 MΩ), and mounted in side-by-side diffusion cells (PermeGear, Inc., Hellertown, PA) with the stratum corneum (SC) facing the receptor chamber. The *in vitro* glass side-by-side diffusion cell apparatus as described by Tang et al. [16] was used. The cells were maintained at 32 °C by a controlled temperature water circulator.

The specific conductivities of the skin samples were determined by a four-terminal resistance method as described by LaCount and Kasting [17] and later modified for side-by-side diffusion cells by Baswan et al. [18]. The cells were filled with PBS + 0.02% NaN_3 and allowed to equilibrate for at least 1 h. Ag/AgCl electrodes, made by the authors with bare silver wire (A-M Systems, Sequin, WA) and 0.1 N hydrochloric acid (Thermo Fisher Scientific, Waltham, MA) were used. Driving electrodes were placed in series with the skin sample, an Agilent Model 33220A waveform generator (Agilent Technologies, Santa Clara, CA), a 10.01 kΩ (±0.02%) precision standard resistor (to establish a reference voltage), and a 1 MΩ resistor (to limit current in the circuit). A square wave signal, 0.1 Hz and 10 V_{p-p}, was generated by the waveform generator, and the voltage drop across the sensing electrodes was measured with a Tektronix model TBS1064 oscilloscope (Tektronix, Beaverton, OR). Utilizing two channels of the oscilloscope allowed for the procurement of the voltage across the skin and the 10.01 kΩ resistor simultaneously, and the ratio of the voltage drops multiplied by 10.01 kΩ yielded the skin resistance. The use of the low magnitude, low frequency signal ensured the measurement had little impact on tissue electrical properties [17,18] and was primarily resistive in nature. Conductivity measurements were used to test for skin integrity and also to create a randomized block design for the various iontophoretic current regimens according to Kasting et al. [19]. Skin conductivity was also measured periodically throughout the experimental procedure.

The skin samples were allowed to equilibrate in PBS + 0.02% NaN_3 overnight, allowing the skin to become fully hydrated in the buffer solution. Skin conductivities were re-measured on the morning of the study to confirm that overnight hydration did not lead to significant changes in the skin. Previous experience with human skin maintained under these conditions has shown that good barrier function is maintained over a period of at least 48 h (see [17] and the Supplemental material associated with this article). On the morning of the study, the donor chambers of the cells each received 1.67 μCi mL^{-1} of radiolabeled cortisol. The chemical concentration (18 nM) was below the 83 nM lower end of the normal range of cortisol concentration in the blood [3]; it was chosen to ensure the lower end of the blood cortisol concentration could be detected. Ethanol was used as the solvent for the radiolabeled material, but because the concentration of ^{3}H-cortisol was so low, the donor chamber contained <0.2% ethanol. This concentration of ethanol is far below those demonstrated to influence skin permeability [20].

Each experiment involved four experimental stages, as adapted from Sims et al. [13]. Stage I was a first run of passive transport, before any electric current was applied, and lasted 4–24 h. During stage II, a fixed current was applied across the skin. Skin resistance was monitored throughout this stage with the Tektronix oscilloscope. Stage II lasted for three hours for all replicates. At the end of stage II, the current was turned off. Stage III was a second run of passive transport which took place in the 3–4 h immediately after turning off the current. Stage IV was a third run of passive diffusion which took place the day after the current was turned off (18–21 h after ending the current). Aliquots (0.75 mL) were collected periodically from receptor chambers and replaced with fresh buffer solution throughout the four stages. Receptor samples were analyzed for ^{3}H in Ultima Gold XR™ cocktail using a Tri-Carb 2900TR Liquid Scintillation Analyzer. Fluxes determined for each stage were calculated only after ^{3}H-cortisol had reached steady-state flux (see Section 3.1). Thus, because ^{3}H-cortisol achieved steady-state within one hour of the beginning of each stage, the lengths of time of stages I, III, and IV do not affect the results.

Four electrical stimulation regimens were tested in this study, along with passive diffusion as a point of comparison. To be consistent with the iontophoresis literature, we will use the term “cathodal polarity” for the condition in which the cathode is placed on the epidermal side of the skin and the anode is placed on the dermal side. The term “anodal polarity” then describes the opposite polarity configuration in which the anode and cathode are switched. Cathodal polarity thus corresponds to what is elsewhere termed “reverse iontophoresis” [8]. In this study we aimed to examine the transport of cortisol from the blood to the surface of the skin. Thus, contrary to a standard drug delivery experiment, ^{3}H-cortisol was applied on the dermal side of the skin (in the donor chamber) and extracted on the epidermal side of the skin (in the receptor chamber). In the cathodal polarity configuration regimens included: 113 μA cm^{-2} (200 μA), 56 μA cm^{-2} (100 μA), and 28 μA cm^{-2} (50 μA). In the anodal polarity configuration, 113 μA cm^{-2} (200 μA) was tested. The current regimens examined in this study were all applied across the same area (1.77 cm^2); thus current density and total current are proportional. Ag/AgCl electrodes as described above were used to generate the electric field and deliver the current during stage II. These electrodes were placed in series with three skin replicates and a power source. For the 113 μA cm^{-2} and 56 μA cm^{-2} regimens, the power source was a Trivarion ActivaDose II Phoresor Unit (ActivaTek, Inc., Salt Lake City, UT), which provided a constant, controlled current for the duration of stage II. For the 28 μA cm^{-2} regimen, a Wilson current mirror circuit designed and built by the Novel Devices Lab in the College of Engineering and Applied Sciences at the University of Cincinnati (Cincinnati, OH) was employed. This smaller circuit based

on a quad PNP silicon transistor package (Serial #MPQ3798,99, Central Semiconductor Corp., Hauppauge, NY) was placed in series with the skin and the power source to provide the desired electrical current. The power source for this regimen was a TDK-Lambda dual regulated power supply Model LQD-422 (TDK-Lambda, National City, CA). The electrical current through the skin was carefully monitored by measuring the voltage drop across a standard resistor in series with the skin. This procedure ensured that the current remained constant throughout stage II.

3. Data collection and analysis

3.1. Calculations

The electroosmotic water flow induced by the application of an electric field across the skin carries uncharged molecules in the anode-to-cathode direction. Thus, if the anode is placed on the dermal side of the skin, water flow will enhance the flux of uncharged molecules from the dermal to the epidermal side of the skin. An enhancement factor has been defined as the ratio of the flux from the applied electric field to the passive flux as follows [13]:

$$E = \frac{\textit{flux with applied current}}{\textit{passive flux}} \tag{1}$$

For the condition described above, where the anode is the placed in the donor chamber, $E > 1$. If the cathode is placed in the donor chamber, thus reversing the polarity of the electric field, $E < 1$ [13]. Enhancement factors were calculated for each skin replicate individually and then averaged across each regimen, so as to use each skin replicate as its own control. A value of E was determined for each passive transport run, resulting in three different E values for each regimen: stage II/I, stage II/III, and stage II/IV.

Flux over the time interval t_1 to t_2 was calculated from the data as follows:

$$J = \frac{\Delta M(t)}{\Delta t} \tag{2}$$

where $\Delta M(t)$ is the cumulative mass of ^{3}H-cortisol equivalents permeated in DPM cm^{-2} and $\Delta t = t_2 - t_1$ in hours. Flux was calculated at each time point and then averaged across each stage after steady-state flux was achieved, giving an average flux for each stage and current regimen.

Permeability coefficients, k_p, were also calculated for each stage and current regimen as follows:

$$k_p = \frac{J_{ss}}{\Delta C_v} \tag{3}$$

where J_{ss} was the steady-state flux in DPM cm^{-2} h^{-1} and ΔC_v was the concentration difference of the radiolabeled probe external to the SC membrane in DPM cm^{-3}. Steady-state fluxes were determined by finding the slope of the linear portion of a plot of cumulative permeation $M(t)$ *vs*. time and dividing by the exposed area of the skin sample (1.77 cm^2). The k_p values were useful for comparing passive permeability determined in these experiments with literature values; under iontophoresis conditions they clearly become functions of the electric field and associated current.

3.2. Statistical analysis

Statistical analysis was carried out using SigmaPlot. An analysis of variance performed for each experiment and each regimen revealed that there were no significant differences in flux results across the skin donors. The skin obtained from each donor was evenly divided across experimental regimens. The number of replicates, n, was 30 for the passive regimen, 6 for the 28 μA cm^{-2} regimen, 15 for the 56 μA cm^{-2} regimen, 17 for the 113 μA cm^{-2} cathodal regimen, and 5 for the 113 μA cm^{-2} anodal regimen. In each experimental setup, 3 of the 5 regimens were tested, each with 3 replicates (total $n = 9$ per experiment).

Statistical comparisons between ^{3}H-cortisol flux during each stage for each regimen were made by Kruskal-Wallis one way analyses of variance on ranks, as the data failed normality tests (Shapiro-Wilk). Flux distributions for each regimen were positively skewed and are presented as medians with upper and lower interquartile ranges, with individual data points representing outliers.

4. Results

4.1. Cumulative ^{3}H-cortisol diffusion over time

Typical results of a ^{3}H-cortisol passive transport experiment are shown in Fig. 1. Steady-state flux of ^{3}H-cortisol was achieved within the first hour post-dose.

Typical results of a ^{3}H-cortisol transport experiment during which 113 μA cm^{-2} was applied through the membrane in the cathodal polarity are shown in Fig. 2A. This current regimen enhanced the flux of ^{3}H-cortisol across the membrane, as evidenced by the increase in slope from stage I to stage II. The average enhancement factor (stage II/stage I) for this regimen was found to be 7.3 ± 1.9 (Table 1). The iontophoretic flux remained higher than the passive flux in the hours immediately after the current was turned off (stage III), but was not as high as during stage II. The flux dropped even more the day after the current was turned off (stage IV), returning to the passive rate.

Typical results of a ^{3}H-cortisol transport experiment during which 56 μA cm^{-2} was applied through the membrane in the cathodal polarity are shown in Fig. 2B. This regimen was also found to enhance the flux of ^{3}H-cortisol across the membrane, but not as much as the 113 μA cm^{-2} regimen. The average enhancement factor (stage II/stage I) was found to be 3.0 ± 0.8 (Table 1). This flux enhancement continued throughout stage III, but returned to the passive rate the day after the current was turned off (stage IV).

4.2. Polarity effects

The electrode polarity effects can be seen by comparing the flux values for the 113 μA cm^{-2} regimens to the passive regimen during

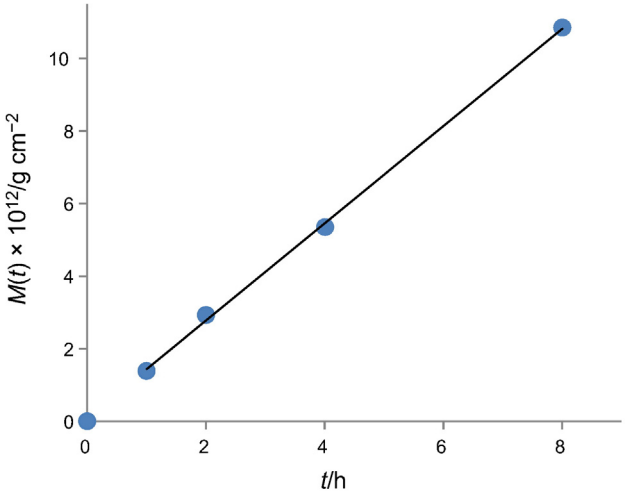


Fig. 1. Typical results of a ^{3}H-cortisol passive transport experiment. The line represents the linear regression of the steady-state portion of the plot, with the slope of the line equal to the steady-state flux. In this example, the slope is 1.3 ($R^2 = 0.9993$), giving a passive flux of 1.3×10^{-12} g cm^{-2} h^{-1}. For this sample, $\Delta C = 6.2 \times 10^{-9}$ g cm^{-3}, yielding a permeability coefficient of 2.1×10^{-4} cm h^{-1}.

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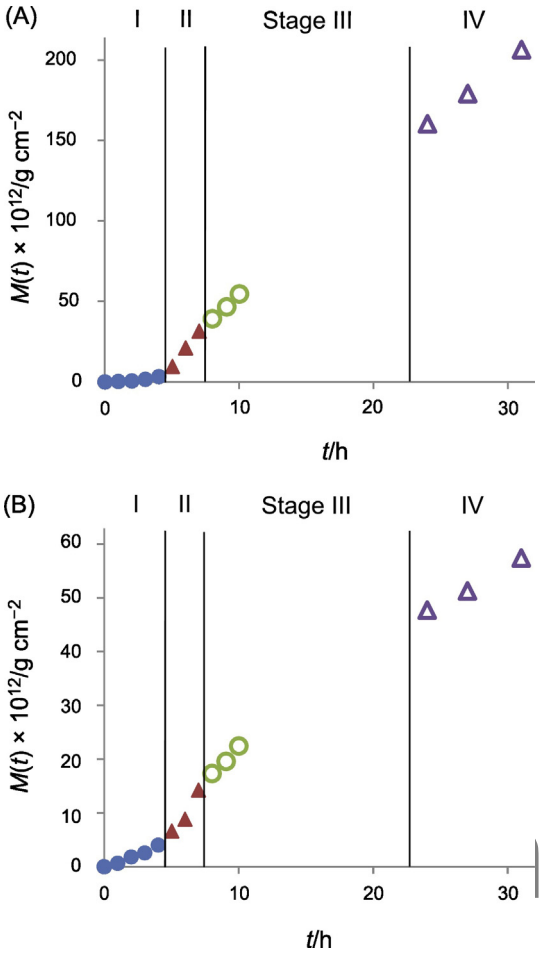


Fig. 2. Typical results of a ^{3}H-cortisol transport experiment during which (A) 113 μA cm^{-2} was applied through the membrane in the cathodal polarity and (B) 56 μA cm^{-2} was applied through the membrane in the cathodal polarity. Note the difference in the y-axes. (●) stage I; (▲) stage II; (○) stage III; (△) stage IV.

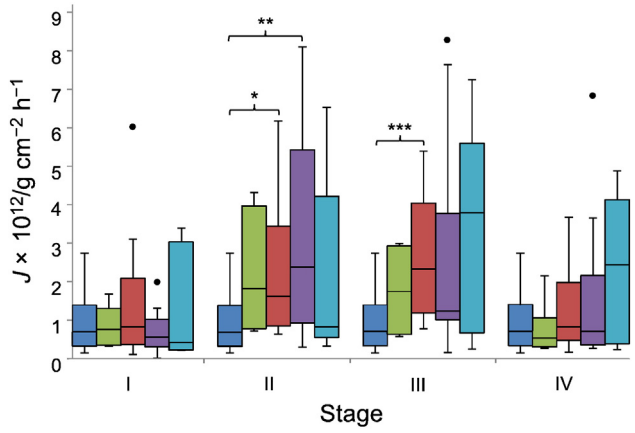


Fig. 3. ^{3}H-cortisol flux, *J*, through human skin during each stage for each current density, *j*. The data are presented as medians with upper and lower interquartile ranges, and the individual data points represent outliers. (■) 0 μA cm^{-2}; (■) 28 μA cm^{-2}; (■) 56 μA cm^{-2}; (■) 113 μA cm^{-2} cathodal; (■) 113 μA cm^{-2} anodal. *p < 0.05, **p < 0.01, ***p < 0.001.

stage II (Fig. 3). In the cathodal polarity, the application of 113 μA cm^{-2} resulted in significantly higher fluxes than did passive diffusion. In the anodal polarity, there was no difference between the 113 μA cm^{-2} and the passive regimens.

4.3. Flux results at each stage

In stage I, there was no significant difference in ^{3}H-cortisol flux from any of the current regimens (Fig. 3). This was expected since during this stage, the electric field had not yet been applied. The overall average flux, *J*, of ^{3}H-cortisol through human skin before any current was applied was found to be $(1.06 \pm 0.15) \times 10^{-12}$ g cm^{-2} h^{-1}, corresponding to a permeability coefficient $k_p = (1.43 \pm 0.21) \times 10^{-4}$ cm h^{-1}.

During stage II, the period during which current was applied, there was a significant difference in ^{3}H-cortisol flux between the passive regimen and the 113 μA cm^{-2} cathodal regimen ($p = 0.003$), as well as between the passive regimen and the 56 μA cm^{-2} cathodal regimen ($p = 0.034$) (Fig. 3). The applications of 28 μA cm^{-2} in the cathodal polarity and 113 μA cm^{-2} in the anodal polarity did not cause a significant change on the transport of ^{3}H-cortisol across human skin during stage II (Fig. 3).

In stage III, the hours immediately after the current was turned off, there was a significant difference in ^{3}H-cortisol flux between the passive regimen and the 56 μA cm^{-2} regimen ($p = 0.001$) (Fig. 3). The difference in ^{3}H-cortisol flux between the passive regimen and the 113 μA cm^{-2} cathodal regimen approached significance ($p = 0.055$). There was no significant difference in ^{3}H-cortisol flux between the passive regimen and the 28 μA cm^{-2} regimen, nor between the passive regimen and the 113 μA cm^{-2} anodal regimen during stage III. After the current had been turned off for 18–21 h, in stage IV, there was no difference in ^{3}H-cortisol flux between the passive regimen and any of the iontophoretic regimens (Fig. 3).

Table 1
Enhancement factors, *E*, for ^{3}H-cortisol transport through human skin determined at each current density, *j* (mean ± SE).

	E		
j/μA cm^{-2}	Stage II/I	Stage II/III	Stage II/IV
28	4.1 ± 1.8	1.4 ± 0.4	4.3 ± 1.9
56	3.0 ± 0.8	0.9 ± 0.1	2.7 ± 0.7
113 cathodal	7.3 ± 1.9	1.9 ± 0.4	4.3 ± 1.5
113 anodal	1.9 ± 0.8	0.9 ± 0.4	1.6 ± 0.7

4.4. Current density vs. permeability coefficient

The average permeability coefficient, k_p, of ^{3}H-cortisol from passive diffusion was found to be $(1.43 \pm 0.21) \times 10^{-4}$ cm h^{-1} in the present study. This value falls within the range of cortisol k_p values found in the literature [20,21]. Permeability coefficients of ^{3}H-cortisol were also calculated for each current regimen during stage II, and are shown in Fig. 4. For the cathodal polarity stimulations, k_p values determined in stage II were found to increase linearly with increasing current density. This was a strong positive correlation ($R^2 = 0.996$) between current density and k_p. The k_p determined for the anodal polarity stimulation was not different from the passive k_p, further showing how the electrode polarity affected ^{3}H-cortisol transport.

4.5. Skin barrier alteration

Skin resistivities were measured before and throughout each experimental procedure to ensure that cortisol flux enhancement was not primarily a result of membrane alteration. Skin samples which did not receive iontophoretic stimulation showed constant resistivities throughout the experiments. Those that did receive iontophoresis dropped in resistivity by varying amounts, depending on initial resistivity. For example, in experiment 3, one skin sample with an initial resistivity of 88 kΩ cm^2 fell to 21 kΩ cm^2 by the end of a 3 h iontophoretic application at 113 μA cm^{-2}. Another skin sample, which received the same current regimen but had an initial resistivity of 27 KΩ cm^2, fell

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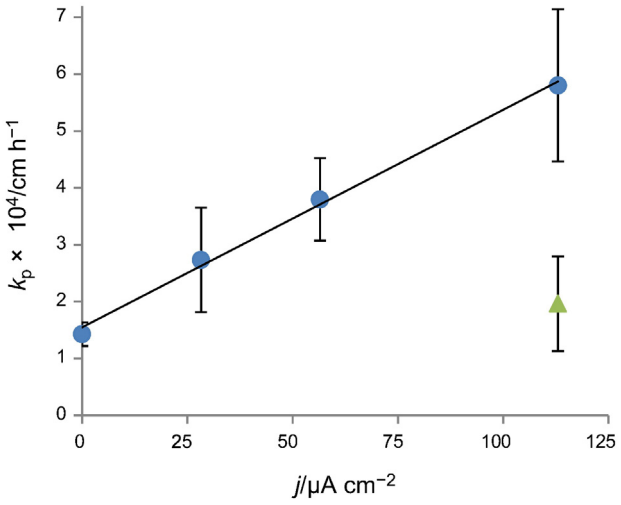


Fig. 4. Permeability coefficients, k_p, of ^{3}H-cortisol determined during stage II at each current density, j. (●) cathodal; (▲) anodal. There was a strong positive correlation ($R^2 = 0.996$) between ^{3}H-cortisol permeability coefficients and current density for those applied with the cathodal polarity.

to only 10 KΩ cm^2 in this time frame. A strong positive correlation between initial skin resistivity and change in skin resistivity pre- to post-iontophoresis was observed with a correlation coefficient of 0.990 (results not shown). That is, the higher the initial skin resistivity, the more it fell during the period of iontophoretic stimulation. On average, the initial skin resistivity was found to be (124 ± 14) kΩ cm^2 ($n = 73$). For those skin samples that received iontophoresis, the final resistivity was found to be (16 ± 3) kΩ cm^2. Even the final value is higher than most cutoff values for identifying damaged skin presented in the literature [22].

5. Discussion

5.1. Cortisol extraction through the skin by reverse iontophoresis in vitro

Presently, monitoring of human cortisol levels requires hospital admission and/or shipping blood/saliva samples to a diagnostic laboratory, which can take 8–10 days to return results [1]. In this study, we examined the feasibility of extracting cortisol across the skin using iontophoresis to enhance its transport, which would allow for continuous and real-time monitoring of cortisol levels. It should be noted that in a wearable device the skin would be exposed to a gel for a prolonged time thus partially hydrating the skin; however, in a realistic scenario, the skin would not be hydrated as extensively, or for as long as it was in these experiments.

Cortisol is a lipophilic compound which can passively diffuse through the skin. Published passive permeability coefficients range from 1.6×10^{-5} to 2.3×10^{-4} cm h^{-1} [20,21] (a value of 6×10^{-6} has been questioned as being an order of magnitude too low [23]); the value determined in this study was $(1.43 \pm 0.21) \times 10^{-4}$ cm h^{-1} (Fig. 4). We examined iontophoretic stimulations starting at 113 μA cm^{-2} in the cathodal direction and ranging downward in a geometrical progression in order to determine the threshold level for distinguishing an electroosmotic cortisol flux from passive flux. For a three hour iontophoretic application, the threshold level was determined to be between 28 and 56 μA cm^{-2}. The electroosmotic flux during stage II reached steady-state values within 0–30 min, most commonly 20–25 min, so it is reasonable to anticipate that the same application period might be required to accurately infer cortisol levels in the interstitial fluid in the epidermis from electroosmotic cortisol levels. It is clear that a reliable kinetic model relating these various concentrations will be an important part of skin biosensor development. Furthermore, it can reasonably be concluded that minimization of the delay between blood and biosensor cortisol (or other systemic biomarker) levels is a design objective for wearable devices. We think, for cortisol, that significant reductions in this delay can be achieved by pursuing extraction mechanisms other than electroosmosis. An alternative approach having such potential is to measure cortisol levels in sweat.

In future work of this nature on human subjects *in vivo*, it will be important to infer interstitial cortisol levels, and the associated blood cortisol levels, in the presence of an outward-directed cortisol flux arising naturally from passive diffusion. The passive flux will itself vary in a manner related to the history of interstitial and blood cortisol levels in the subject. It is clear that a well-designed pharmacokinetic model relating these quantities will be required in order to properly interpret electrically-induced cortisol flux at the skin surface. This was also true for glucose monitors, but the passive background for cortisol is much higher than that for glucose due to its greater skin permeability. Hence, the speed of the iontophoretic response is paramount, and it should be compared with that available from alternative cortisol extraction methods (*e.g.* sweat elicitation) before committing to development of the electroosmotic approach.

5.2. Device integration

Technology integration for continuous sensing is speculated upon in this section. Colorimetric sensors for cortisol, based on antibodies, are readily available but are one-time use without complex microfluidics. Unfortunately, continuous sensors are the most difficult to construct, with few commercial successes such as those enzymatic sensors used in glucose monitoring [24]. Even if robust enzymatic sensors were available for cortisol, at the low concentrations of total cortisol (83 nM [3]) the enzymatic signal would likely be too low. Of the few options available, electrochemical aptamer sensors are potentially attractive since such sensors are label- and reagent-free and stable in biofluids as complex as whole blood [25].

However, aptamer-based sensors often have limitations in sensitivity, especially for small molecules like cortisol (typically high nM to μM limits of detection [26]). In this work, with an initial concentration slightly below the normal range in blood, the passive flux of ^{3}H-cortisol was estimated to be $(1.1 \pm 0.15) \times 10^{-12}$ g cm^{-2} h^{-1} (or 3.1×10^{-15} mol cm^{-2} h^{-1}). If that flux were slowed down using a membrane (such as those used in water filtration which still pass small ions), a concentration would build up near such a membrane. A sensor array could then be placed near the membrane which would concentrate the cortisol. For example, for every cortisol sampling event, a flux of cortisol could be enabled by reverse iontophoresis and the flux maintained until cortisol concentrations fell within the linear range of a cortisol sensor. If once the duty cycle for reaching the proper cortisol concentration was determined, that duty cycle for each pulse of reverse iontophoresis could be the same for each sampling event, and this could provide information on change in cortisol levels from sampling event to sampling event. Perhaps even such a device could be calibrated with a blood or saliva sample for more quantitative measures. Alternately, a reference sensor and analyte, such as for DHEA could be measured as well, and cortisol/DHEA ratio reported [27].

6. Conclusions

Compared to passive diffusion alone, the applications of 113 and 56 μA cm^{-2} DC with the cathode placed on the skin surface were found to significantly enhance the extraction of ^{3}H-cortisol from beneath the skin during the periods of reverse iontophoresis. These fluxes were higher in the hours immediately after the current was turned off, and dropped back to the passive rate by the next day. The application of 28 μA cm^{-2} did not have a significant impact on ^{3}H-cortisol flux across the skin; however, the fluxes resulting from this current regimen were similar in magnitude to those from the 56 μA cm^{-2} regimen. The threshold for current density required to significantly enhance the flux

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of ^{3}H-cortisol across the skin is consequently between 28 and 56 μA cm^{-2}. A reverse polarity regimen of 113 μA cm^{-2} showed little impact on the flux of ^{3}H-cortisol across the skin, establishing that electroosmosis is the primary driving force for the flux enhancements observed in these experiments.

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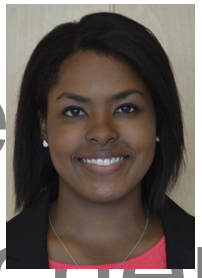
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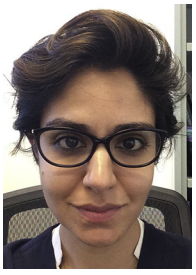
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