I, Eliza C. S. Wilder, hereby submit this original work as part of the requirements for the degree of Master of Science in Pharmaceutical Sciences.

It is entitled:
Human Subjects Testing of Sweat Stimulation Technologies

Student's name: Eliza C. S. Wilder

This work and its defense approved by:

Committee chair: Gerald Kasting, Ph.D.

Committee member: Trudy Gaillard, Ph.D.

Committee member: Kevin Li, Ph.D.
Human Subjects Testing of Sweat Stimulation Technologies

A thesis submitted to the
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by

Eliza C. S. Wilder

B.S. Chemistry, University of Cincinnati

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Committee Chair: Gerald B. Kasting, Ph.D.
Abstract

Human eccrine sweat is a naturally occurring salty biofluid produced in the thermoregulation of the human body. Though most predominantly associated with cooling of the body eccrine sweat has also been used in the diagnosis of diseases such as cystic fibrosis. In cystic fibrosis diagnosis pilocarpine iontophoresis is used at hospitals and clinics nationwide as the gold-standard test to induce sweating on the forearm of infants. Detection of elevated levels of chloride ions in the sweat leads to a positive read for cystic fibrosis.

Along with chloride and sodium ions, sweat has been found to contain numerous other electrolytes, small and large molecules, peptides and proteins which are shown to correlate to other body fluids such as blood, blood derivatives, saliva and tears in levels ranging from mM to pM concentrations. Examination of sweat analytes leads to the potential for noninvasive diagnostic techniques to be developed in the form of on-skin patches which may allow for biomonitoring for conditions other than cystic fibrosis.

After building a database of numerous analytes, their concentrations in sweat and means of partitioning pathways into the sweat glands it was important to begin in vivo assessment of human sweat. The cystic fibrosis sweat test using two iontophoretic devices from Wescor Inc. was performed on 18 healthy subjects at the University of Cincinnati upon approval from the Institutional Review Board (IRB) to examine sweat rates, electrolyte concentrations and effects of multiple doses on the same site. Results indicated a statistically significant difference in sweat rates of men versus women, as well as notable differences in electrolyte concentrations between left arms and right arms of subjects. Subjects who were included in repeat tests on identical sites showed significant decreases in sweat rates.

Novel sweat sensor patches are currently being tested as part of exploratory studies examining alternative methods of sweat stimulation and sensing. Based on the results gained from testing with the commercially available cystic fibrosis testing devices new sweat sensor
technologies can work towards alleviating the current limitations of sweat as a biofluid and allow for broadening the spectrum of sweat biomonitoring.
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I want to thank Dr. Trudy Gaillard for both serving on my committee and for acting as PI on the many human subjects testing studies. She was instrumental in beginning the clinical work for our team. I also would like to thank Dr. Kevin Li for serving on my committee, as well as providing countless valuable insights both in the classroom and outside it. I must also give my utmost appreciation and thanks to Dr. Jason Heikenfeld and all my engineering colleagues without whom this work would neither exist nor have reached its full potential. Dr. Heikenfeld consistently encouraged me to ask questions and constantly inspired me with his infectious enthusiasm.

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Finally I extend my infinite gratitude to my family for their unconditional love, support through the most trying of times and for never doubting me even when I doubted myself. This work is dedicated to them.
# Table of Contents

ABSTRACT .................................................................................. i
ACKNOWLEDGEMENTS .................................................................. iv
TABLE OF CONTENTS ...................................................................... v
LIST OF TABLES .................................................................................. vi
LIST OF FIGURES ................................................................................ vi
(1) INTRODUCTION ................................................................................ 1
(1.1) ULTRASTRUCTURE OF THE ECCrine SWEAT GLAND ......................... 1
(1.2) SWEAT SECRETION AND mM-pM SOLUTE PARTITIONING .................... 3
(1.3) CURRENT SWEAT STIMULATION AND SENSING TECHNOLOGIES......... 10
(2) HYPOTHESIS AND SPECIFIC AIMS .................................................. 13
(3) AN EXAMINATION OF INTRA- AND INTERSUBJECT SWEAT VARIATIONS USING COMMERCIALY AVAILABLE SWEAT STIMULATION TECHNOLOGIES .......................... 14
(3.1) INTRODUCTION ............................................................................ 14
(3.2) MATERIALS AND METHODS ......................................................... 15
(3.2.1) PARTICIPANT DEMOGRAPHICS AND ELIGIBILITY ...................... 15
(3.2.2) NANODUCT® IONTOPHORESIS .................................................... 16
(3.2.3) MACRODUCT® IONTOPHORESIS .................................................. 17
(3.2.4) STATISTICAL ANALYSIS ............................................................. 18
(3.2.5) STUDY APPROVAL AND INFORMED CONSENT ......................... 18
(3.3) RESULTS ........................................................................................ 18
(3.3.1) NANODUCT® ............................................................................. 18
(3.3.2) MACRODUCT® ........................................................................... 19
(3.3.3) DUPLICATION TESTS ................................................................. 20
(3.4) DISCUSSION .................................................................................... 21
(3.5) ETHICAL APPROVAL ..................................................................... 23
(3.6) CONFLICT OF INTEREST .............................................................. 23
(4) EXPLORATORY STUDIES .................................................................. 24
(4.1) IONTOPHORETIC SUDOMOTOR AXON REFLEX PATCH .......................... 24
(4.2) EXERCISE PATCH ........................................................................ 26
(5) CONCLUSIONS AND RECOMMENDATIONS .................................. 28
BIBLIOGRAPHY ................................................................................... 31
APPENDIX A: TESTING THE MICROFLUIDICS OF ON-SKIN TECHNOLOGIES ... 34
APPENDIX B: TESTING OF A NOVEL IONTOPHORETIC SWEAT STIMULATION PATCH .......................................................................................................................... 42
APPENDIX C: TESTING OF NOVEL ON-SKIN SWEAT PATCH THROUGH METABOLIC SWEAT INDUCTION ................................................................................. 47
List of Tables

Table 1 Dimensions and additional physical descriptors for eccrine sweat glands. ..................3
Table 2 Typical concentration ranges for common biomarkers in sweat versus blood, plasma and serum ..........................................................9

List of Figures

Figure 1 Illustration of the secretory coil and dermal duct .........................................................1
Figure 2 Sweat analyte concentration compared to sweat rate ....................................................11
Figure 3 Macroduct® Sweat Collection Spirals and Nanoduct® Neonatal Sweat Analysis System .................................................................................................15
Figure 4 Intra- and interpersonal Nanoduct® average values ..................................................19
Figure 5 Intra- and interpersonal Macroduct® sweat rate values .................................................20
Figure 6 Duplication tests performed by the Macroduct® device .............................................21
Figure 7 Schematic of QSART iontophoretic patch .................................................................25
1 Introduction

1.1 Ultrastructure of the Eccrine Sweat Gland

The human eccrine sweat gland comprises several geometrically distinct portions, the secretory coil, the dermal duct, and the upper coiled duct, as shown in Figure 1. The upper coiled duct is also often referred to as the acrosyringium. In this region, the sweat duct expands in diameter slightly, until it emerges at the surface of the skin (1-3).

![Cross-sectional and top-down illustration of the (a) secretory coil and (b) dermal duct.](image)

**Figure 1** Cross-sectional and top-down illustration of the (a) secretory coil and (b) dermal duct.

Typical ranges for the physical dimensions of the human eccrine sweat gland are shown in Table 1. These dimensions also have the potential to vary due to differences in gender, age, and race (1, 2, 4). The largest portion of the eccrine sweat gland is the secretory

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1 Sections 1.1 and 1.2 are quotations from the published review paper “The microfluidics of the eccrine sweat gland, including biomarker partitioning, transport, and biosensing implications” by Sonner, Wilder et al (2015). A more comprehensive overview of the computational modeling and analyte partitioning can be found in the full manuscript which can be accessed online at http://scitation.aip.org/content/aip/journal/bmf/9/3/10.1063/1.4921039.

coils, which can be upwards of 700 µm in diameter in its natural bundled state and with an extended length of several millimeters (2). The inner-diameters of the secretory coil and ductal regions are referred to as the lumen, ranging from 5µm to 10s of µm (2, 5, 6).

Top-down cross-sectional illustrations of the secretory coil and dermal duct are shown in Figure 1. These cross-sections are important, as they are the interface through which biomarkers partition into new sweat. As shown in Figure 1(a), the secretory coil contains three primary cells: the clear (secretory), dark (mucoid), and myoepithelial (supportive) cells (3, 6, 7). In comparison, the dermal duct contains only a bilayer of basal and luminal cells (6, 7) as shown in Figure 1(b). Clear cells are hypothesized to be the dominant source of sweat secretion due to the abundance of mitochondria present within the cell (7). Dark cells, however, are believed to be involved in membrane-cellular transport and may also have the ability to act as clear cells, themselves (7). Myoepithelial cells are thought to act as a supportive network between the basolateral membrane (7). Regarding the dermal duct, the luminal cells form a semi-cuticular border with many microvilli protruding into the lumen of the duct, which increases the effective surface area of the cells for absorption or secretion (7). The basal cells in the dermal duct are characterized with abundant mitochondria, also suggesting a highly active role in sodium reabsorption (3). Other physical descriptors of sweat glands and their densities across the body are listed in Table 1.
Table 1 Dimensions and additional physical descriptors for eccrine sweat glands (1, 2, 6, 8-11).

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Value (µm)</th>
<th>Descriptor (units)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Secretory Coil</strong></td>
<td></td>
<td>Sweat surface tension (mN/m)</td>
<td>71.8</td>
</tr>
<tr>
<td>Length</td>
<td>2000-5000</td>
<td>Composition of sweat (% water)</td>
<td>99</td>
</tr>
<tr>
<td>Outer diameter</td>
<td>60-120</td>
<td>Maximum hydrostatic pressure (kN/m²)</td>
<td>70</td>
</tr>
<tr>
<td>Inner diameter</td>
<td>5-40</td>
<td>Typical sweat rate range (nL/min/gland)</td>
<td>&lt;1-20</td>
</tr>
</tbody>
</table>

| **Dermal (straight) duct**  |            |                                  |       |
| Length                      | 2000       |                                  |       |
| Outer diameter              | 50-80      | **Approximate eccrine sweat gland densities** |       |
| Inner diameter              | 10-20      | **Location**                     |       |
|                            |            | **Gland density (cm²)**          |       |
| Upper coiled duct (Acrosyringium) |        | Palm, finger                     | 250   |
| Length                      | 200-300    | Forehead, forearm                | 150   |
| Outer diameter              | 120        | Abdomen, back, legs              | 100   |
| Inner diameter              | 20-60      | Number of glands/individual (10⁶) | 1.6-5 |
|                            |            |                                  |       |
| **Total bundled** (coiled) diameter | 500-700 |                                  |       |

1.2 Sweat secretion and mM-pM solute partitioning

The partitioning of Na⁺ and Cl⁻ ions, the most abundant solutes in sweat, is an active process which is also fundamental to the secretion of water (11, 12). Water and NaCl are partitioned from blood into the lumen of the secretory coil through seven general steps as outlined by several authors (11-13): (1) signals from the brain stimulate cholinergic nerve endings surrounding the sweat gland (due to stress, temperature, or other known stimuli (11, 14)), (2) stimulation causes Ca²⁺ influx into the surrounding cells, (3) Ca²⁺ stimulates K⁺/Cl⁻ channels causing Cl⁻ to enter the lumen and K⁺ to exit the luminal cells on the basolateral membrane, (4) this exiting of K⁺ and Cl⁻ causes a chemical potential gradient permitting co-transport of Na⁺-K⁺-2Cl⁻ into the cell, (5) K⁺ and Na⁺ recycle across the basolateral membrane permitting increased flux of Cl⁻ into the lumen, (6) once electric field builds, Na⁺ enters the lumen via intracellular junctions to re-establish charge neutrality, and (7) the lumen then has greater
osmolality (NaCl) than the surrounding cells which causes an influx of water into the lumen to re-equilibrate. This preliminary fluid then “pumps” along the length of the duct.

Through evolution the body has developed mechanisms to retain electrolytes which would be rapidly lost when large volumes of sweat are used for cooling the body. Once the preliminary sweat in the coil is formed, the osmotically generated pressure forces the fluid up from the coil into the ductal region (3). Sodium and chloride ions are then actively reabsorbed via two channels, the epithelial sodium channel (ENaC) and the CF transmembrane regulator (CFTR), respectively (12). Levels of both Na\(^+\) and Cl\(^-\) are elevated at high sweat rates, indicating that the two channels that reabsorb sodium and chloride ions work at-or-near the same rates regardless of the sweat flow rate in the duct (12, 13). In most instances, chloride concentrations are 20–25 mM less than sodium concentrations due to the contribution of other anions (7) present in sweat such as bicarbonate (HCO\(_3\)). Both sweat rates and Na\(^+\) and Cl\(^-\) concentrations differ across the body due to regional differences in the glands themselves (15).

Potassium partitioning is interesting to examine because although it is a small ion like sodium or chloride, its transport mechanism is vastly different. From an applied perspective, K\(^+\) in plasma predicts muscle activity (16) and a myriad of conditions related to hypo- or hyperkalemia (17). Further, K\(^+\) concentration in sweat is proportional to blood concentration and is independent of sweat rate (18). The precise method of K\(^+\) partitioning into sweat has not been fully confirmed but the concentration appearing at the surface of skin is likely influenced by K\(^+\) leak channels in the duct (11). Kenzo Sato conducted a very detailed analysis of measuring K\(^+\) exiting the secretory coil vs. K\(^+\) exciting the duct onto the surface of skin (3). The results for the preliminary sweat emerging from the secretory coil showed K\(^+\) isotonic to blood (5 mM). However, sweat emerging from the duct was found to be several mM higher in concentration, possibly indicating that the active process of ductal reabsorption
of Na\(^+\) results in possible secretion of K\(^+\) into sweat in the duct. Sato’s experiment was conducted on glands extracted from the palm (not in the body).

Ammonia is of interest in sweat because it has been shown to track with exercise intensity and blood levels (19-21). Ammonia is also of interest because it is a possible and needed substitute for lactate measurement of anaerobic condition. Ammonia (NH\(_3\)) passes through the cells lining the secretory coil by means of a passive diffusive mechanism (3). In the lumen, most ammonia molecules that partition from blood will protonate to ammonium (NH\(_4^+\)) due to the relatively low pH of sweat which approaches a pH of 5 for low sweat rates and increases towards 7 at higher sweat rates (7). Assuming an ammonia pKa of 9.3, the Henderson-Hasselbalch equation then predicts that even at a pH of 7, the vast majority of ammonia (NH\(_3\)) that is able to diffuse into the sweat gland lumen will be protonated into ammonium (NH\(_4^+\)) (22). Therefore, while NH\(_3\) has a high level of diffusivity through cellular membranes, NH\(_4^+\) will not because of its electrical charge (7). This essentially causes “trapping” of ammonium in the lumen of the secretory coil and dermal duct. This supports the discovery that the concentration of ammonium (NH\(_4^+\)) in sweat is amplified (20–50 times higher) compared to the concentration of ammonium in plasma (3).

Ethanol metabolism is well-studied in humans as it is the key molecule which causes intoxication via excessive consumption of alcohol. Several studies show a strong correlation between blood and sweat ethanol concentrations which could potentially enable continuous blood alcohol (BAC) analysis by sweat measurement (23-25). Gamella et al. presented a non-invasive electrochemical bio-sensing device which monitored ethanol concentrations in sweat whilst demonstrating higher sensitivity compared to breathalyzers commercially available at the time in 2014 (23). Furthermore, their device showed a response to sweat ethanol concentrations within only 5 minutes after ingestion of an alcoholic beverage, while also
demonstrating strong correlation to BAC ($r=0.934$) (23). Such capability is available commercially (e.g., a wearable bracelet by SCRAMVR Systems).

Due to the hydrophilic-lipophilic balanced nature of ethanol, this molecule is watersoluble and has the ability to permeate through most membranes within the human body. In many studies on ethanol concentration, correlations between sweat and blood conclude that ethanol is found about 20% more concentrated in sweat compared to blood or plasma (23, 25). However, when comparing the water contents of blood and sweat, one also finds that sweat has approximately 20% more water than that of blood (24). In fact, when comparing the concentration of ethanol in terms of millimoles per liter of water instead of liter of solution (more generalized), one finds that sweat and blood ethanol concentrations correlate one to one (24). This simple result further shows that numerous types of alcohols likely correlate well between sweat and blood. Furthermore, this reinforces that correlations with blood should always consider the fact that blood has a lesser percentage of water compared to sweat.

Cortisol is a vital life hormone released in response to stress from the hypothalamo-pituitary-adrenal axis (26). Cortisol’s lipid-soluble nature allows for passive transport between the lipid-bilayer membrane of cells, allowing for cortisol’s presence to be detected in many bodily fluids including sweat (27, 28). However, there are two distinct structures of cortisol. Namely, unbound cortisol which is able to diffuse through membranes via intracellular passive transport, while cortisol bound to carrier proteins is unable to make this diffusion (28, 29). In saliva, free cortisol is carried by the bloodstream to acinar cells at the ends of the salivary glands where its lipid-soluble nature allows for passive transport into the salivary glands, causing salivary cortisol levels to be independent of salivary rates (27). Studies also show that saliva cortisol correlates with serum unbound cortisol levels (29).
This mechanism of unbound cortisol partitioning and salivary rate independence found in salivary gland function can be speculated to be true for sweat glands as well. Cortisol levels in sweat have been recorded as ranging from $2.21 \times 10^{-5}$ to $3.86 \times 10^{-4}$ mM, with the greatest concentration being found in the morning and correlating with salivary concentrations (26). Comparatively, blood cortisol concentrations typically range from $1.24 \times 10^{-4}$ to $4.0 \times 10^{-4}$ mM (30). It should be noted that type 2 11-b-hydroxysteroid-dehydrogenase (HSD), which is an enzyme capable of converting cortisol to cortisone, has also been detected in cells of the eccrine sweat gland duct (30).

Urea is a nitrogen-containing metabolite typically excreted by the kidney but is found to be alternatively excreted through sweat glands. Urea from sweat is even visibly observable as a white skin crust in patients suffering from kidney failure (31, 32) (with urea levels up to 50 times greater than in serum). Urea partitioning into sweat has not been fully confirmed but ideas of passive transport across glands from blood into sweat precursor fluid has been a common speculation (3, 7, 33) and emphasized by urea’s diffusibility through membranes as well as its ratio relationships to plasma (33). Another theory of urea partitioning is possible metabolism by the sweat glands or other means such as Urea Transporter-B (UT-B) (31, 32, 34). Regardless, urea concentrations in sweat for healthy males are typically in the range from 2 to 6 mM (7, 33), with serum urea concentrations ranging from 5 to 7 mM (34). At low sweat rates, the sweat to plasma urea concentration ratio reaches upper limits of 4 but approach 1 as sweat rates increase (3, 33). Other studies are less conclusive, perhaps due to use of significantly uncontrolled sweat generation and collection methods (34). Further study is clearly needed to determine if clinical data with higher urea concentration at low sweat rates is due to evaporation or other causes.

Lactate is a small molecule metabolite produced by the body as a result of anaerobic activity (high exertion exercise, critically ill patients, etc.) (35). Lactate is used to sustain the
purine nucleotide cycle (PNC) and tricarboxylic acid cycle (TCA cycle) which allows energy production in the absence of adequate oxygen (35). The transport of lactate from plasma to sweat remains not fully understood and inherently complex. Lactate has two likely origins: (1) a flux of the metabolite due to the metabolism of a “hard-working” sweat gland itself and (2) any active or passive transport mechanism which transports the metabolite between plasma and sweat.

What is clear at this point is that sweat lactate is at least an indirect indicator of body exertion (36) and is likely more directly related exertion of the sweat gland itself in response to whole body exertion. Several studies (19, 37, 38) have experimentally investigated the relationship between plasma and sweat lactate levels after exercise, with widely ranging sweat concentrations as low as 6 mM (19) and as high as 100 mM (39).

Various peptides and small proteins have been detected in eccrine sweat and have been shown to correlate with plasma levels. Polypeptides are long linear chains of multiple amino acids found throughout the body (40). Proteins are also chains of amino acids and therefore can be classified as polypeptides, but are typically seen as three-dimensional structures rather than in linear form (40). These structures are much larger than small molecules and their partitioning into sweat is unexpected at first glance. Exact partitioning models remain unknown, but the initial work on sweat correlations with blood is promising.

Cytokines are reduced regulatory proteins synthesized and released by immune system cells as well as a variety of other cells including those in eccrine sweat glands (41). In previous sweat patch studies, interleukin-6 (IL-6), IL-1a, IL-1b, IL-8, tumor necrosis factor-a (TNF-a), and transforming growth factor-b (TGF-b) have been detected directly in eccrine sweat and have been shown to have a direct correlation with plasma levels (41). However, cytokines can also be reproduced systemically or locally (41) at the sweat gland. Many cytokine responses are systemic in cytokine production, such that cytokine production by or
near the sweat gland would mirror cytokine production in the body, such that sweat gland produced cytokines could still correlate with concentrations in blood. Therefore, in some cases, the “source” of the cytokine generation may be less important.

Peptides such as neuropeptide Y (NPY) also display correlation between sweat and blood. NPY is a linear polypeptide found in dermal nerve fibers, sweat glands, and hair follicles (42). NPY is found to exhibit anti-stress activities when receptors are activated, along with numerous other effects such as anti-depressive properties (43). In a study of women with major depressive disorder (MDD) in remission, levels of cytokine and neuropeptide concentrations were measured via sweat patch and plasma comparisons (44). It was displayed that levels of sympathetic NPY increased from 4.45x10^{-10} mM in sweat of healthy patients to 1.19x10^{-10} mM in sweat of MDD patients, with levels comparable to plasma concentrations in both groups (44). This is presumably due to an elevation in stress response due to MDD.

Table 2 Typical concentration ranges for common biomarkers in sweat versus blood, plasma, and/or serum with subscripts indicating particular fluid (b-blood; p-plasma; s-serum) (3, 7, 15, 24, 26, 30, 34, 38, 41, 44-47).

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Partitioning and sweat rate dependent (SWD) or mainly independent (SWI)</th>
<th>Concentration range (mM) in sweat at surface</th>
<th>Concentration range (mM) in blood, plasma, serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (Na+)</td>
<td>Active—SWD</td>
<td>10–100</td>
<td>135–150&lt; subscripts &gt; b</td>
</tr>
<tr>
<td>Chloride (Cl−)</td>
<td>Active—SWD</td>
<td>10–100</td>
<td>96–106&lt; subscripts &gt; s</td>
</tr>
<tr>
<td>Potassium (K⁺)</td>
<td>Passive—SWI</td>
<td>4–24</td>
<td>5–6</td>
</tr>
<tr>
<td>Ammonium (NH₄⁺)</td>
<td>Passive (amplified)—SWI</td>
<td>0.5–8</td>
<td>20–50&lt; sweat concentration&lt; subscripts &gt; p</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Passive—SWI</td>
<td>2.5–22.5</td>
<td>2.5–22.5&lt; subscripts &gt; b</td>
</tr>
<tr>
<td>Cortisol</td>
<td>Passive—likely SWI</td>
<td>2.21x10^{-5}–3.86x10^{-4}</td>
<td>1.24x10^{-4}–4.0x10^{-4}&lt; subscripts &gt; b</td>
</tr>
<tr>
<td>Urea</td>
<td>Various, not confirmed</td>
<td>2–6</td>
<td>5–7&lt; subscripts &gt; s</td>
</tr>
<tr>
<td>Lactate</td>
<td>Generated by gland—SWD</td>
<td>5–60</td>
<td>1–7&lt; subscripts &gt; b</td>
</tr>
<tr>
<td>Neuropeptide Y (NPY)</td>
<td>Various, not confirmed</td>
<td>1.19x10^{-10}–6.82x10^{-10}</td>
<td>1.41x10^{-10}–6.11x10^{-10}&lt; subscripts &gt; p</td>
</tr>
<tr>
<td>Interleukin 6 (IL-6)</td>
<td>Various, not confirmed</td>
<td>2.91x10^{-10}–6.54x10^{-10}</td>
<td>2.15x10^{-10}–5.69x10^{-10}&lt; subscripts &gt; p</td>
</tr>
</tbody>
</table>
As shown in Table 2 many of the molecules in sweat have concentrations which are sweat rate dependent. The beauty of measuring molecules which do exhibit sweat rate dependency is the ability to predict concentration levels in sweat at given sweat rates and vice versa. This trend is critical in the development of novel sweat technologies given that concentrations and sweat rates can be indicative of each other. In current technologies sodium and chloride ions are measured after sweat stimulation to diagnose patients with cystic fibrosis.

1.3 Current Sweat Stimulation and Sensing Technologies

The most widely recognized sweat technology is the Wescor Macroduct® Sweat Collection System which stimulates small volumes of sweat followed by sweat collection for laboratory analysis of chloride concentrations. Sweat chloride testing is widely considered the gold-standard for infantile cystic fibrosis diagnosis (45). Cystic fibrosis is a genetic disease which causes a malfunction in the cystic fibrosis transmembrane regulator channel, found on the sweat duct, and prevents Cl⁻ reabsorption. This leads to thick mucus that coats many of the vital organs in the body, predominantly the lungs, which can result in fatality (48). Because the body is unable to reabsorb chloride ions heightened Cl⁻ levels in sweat are indicative of cystic fibrosis (12) (Figure 2). Sweat chloride values in infants less than 40 mmol/L are negative for cystic fibrosis while those values greater than 60 mmol/L are considered cystic fibrosis positive (49). Healthy adults can exhibit sweat chloride values of greater than 60 mmol/L (49).

In order for hospitals to be able to test sweat chloride levels, sweat must be chemically stimulated. In naturally occurring sweat an increase in temperature causes hypothalamic release of acetylcholine to begin the secretion process. However a technique called “iontophoresis” can also aid in the stimulation of sweat secretion.
Figure 2 Sweat analyte concentrations compared to sweat rate (45). In the absence of cystic fibrosis sweat chloride and sodium levels are expected to increase with sweat rate. However patients who test positive for cystic fibrosis have heightened levels of chloride in sweat across all sweat rates.

Medically speaking, iontophoresis is the process by which charged drugs are delivered through tissues via electric current (50). A drug of choice is placed on two electrodes, one being of the same charge as the drug (stimulation electrode) and one being of opposite charge (return electrode) (50). Typically the positively charged electrode is the anode which will repel a positively charged drug into the skin (51). Alternatively the negatively charged electrode is the cathode which will repel a negatively charged drug into the skin (51). The electrodes are then placed on neutral areas of the body, and are attached to a power source which delivers a predetermined direct (Galvanic) current to the electrodes for a set amount of time (50). The flow of current between the electrodes drives the drug into the site of choice through the tissue (50).

Historically iontophoretic drug delivery has been used in the delivery of drugs ranging from antibiotics, to steroids and local anesthetics (50). One of the more common uses of iontophoretic drug delivery, however, is that of pilocarpine to activate the sweat glands.
Pilocarpine is a drug employed in the stimulation of sweat glands, but is furthermore used in the treatment of glaucoma in the eyes, and also dry mouth by means of stimulating the salivary glands (52). In natural sweat response the brain receives a signal from an increase in localized heat which causes a release of acetylcholine from the brain. This chemical travels the length of the spinal cord, then attaches to muscarinic action sites at the base of the sweat glands to begin secretion (12). Pilocarpine has the same ability to bind to the noted action sites at the base of the sweat glands, yielding a sweat response (52) once it has been driven through the skin with iontophoresis. Acetylcholine is metabolized rapidly by acetylcholinesterase; however pilocarpine is not as susceptible to acetylcholinesterases allowing for a longer sweat response to occur (52). Other cholinergic agonists can produce sweat responses through the described mechanism including methacholine and carbachol though through demonstration of nicotinic affinity (52).

The previously mentioned Wescor Macroduct® device as well as the Wescor Nanoduct® utilizes pilocarpine iontophoresis for sweat stimulation and analysis in hospitals and clinics nationwide. These devices offer the best starting point through which novel sweat stimulation and sensing technologies can stem. Typically the Wescor devices are used solely on patients who are being screened for, or who have previously tested positive for, cystic fibrosis. A study was therefore created and performed to test healthy, non-cystic fibrosis adults to examine sweat variations intra- and interpersonally and to serve as guidance in novel sweat device design.
2 Hypothesis and Specific Aims

It is hypothesized that useful guidance for novel sweat sensor development will be obtained from moderate scale testing of commercial sweat stimulation and sensing devices on healthy subjects. This will be accomplished through three specific aims:

- Using the FDA approved Wescor Macroduct® and Nanoduct® devices recreate the standard cystic fibrosis testing procedure. Analyze the collected sweat through examination of sweat rate, electrolyte concentration, and volume of sweat produced from each subject.

- Evaluate the relationship between sweat electrolyte concentration and sweat rate via sequential application of the two test methods. Compare the relationship to that found in the literature for simultaneous measurements.

- Build a database of intra- and intersubject variation of sweat rates and NaCl concentrations in order to guide sensor development.

The intention of this work is to offer such a tool for researchers to combat the aforementioned sweat limitations and advance the developments of sweat sensing devices for consumer use.
3 An Examination of Intra- and Intersubject Sweat Variations Using Commercially Available Sweat Stimulation Technologies

3.1 Introduction

Human eccrine sweat is a biofluid produced to aid thermoregulation of the body (12) and is found to contain numerous biomarkers (11, 45). Biomarkers in sweat include vital electrolytes such as sodium and chloride, small molecules and a number of peptides (45). Examination of these biomarkers can lead to diagnosis of certain diseases; most commonly, cystic fibrosis as evaluated via sweat chloride concentration. Cystic fibrosis is caused by a malfunction in the cystic fibrosis transmembrane regulator (CFTR) which transports chloride ion across cellular membranes throughout the body (48). Without the resorption of vital chloride ions by the CFTR in the ductal region of the sweat gland, heightened levels of chloride ions can be detected in sweat, thus leading to the diagnosis of cystic fibrosis (45). Testing for cystic fibrosis is performed through iontophoretic delivery of a drug called pilocarpine, which begins the sweat secretion process (49). A chloride concentration of >60 mM provides indication of cystic fibrosis in infants, however, adults can show levels of chloride ions >60 mM and be CF negative (49). Though sweat testing is considered the gold-standard for cystic fibrosis screening, the potential for sweat to be used in other diagnostic arenas is highly desirable (45). Sweat sensor development is becoming increasingly popular; however, more information about intrapersonal and interpersonal sweating variations needs to be studied. Using the commercially available cystic fibrosis sweat testing devices, Wescor Macroduct® and Nanoduct®, the investigators set out to examine intrapersonal and interpersonal variations of Na⁺/Cl⁻ concentration and sweat rates for several subjects via pilocarpine iontophoresis. Images of these devices are shown in Figure 3. Analysis included variation between left and right arms, gender, and sweat rates after multiple dosages applied on the same site several weeks apart.
3.2 Materials and Methods

Each subject was asked to schedule two one-hour appointments, at minimum 24 hours apart. During the first appointment the Nanoduct® procedure was performed; subsequently the Macroduct® test procedure was performed at the second appointment. Upon arrival, subjects were equilibrated to the testing room for at least 20 minutes. The rooms in which testing occurred were maintained at an average temperature of 22.7°C and 45% relative humidity to ensure subject sweating was purely from iontophoresis rather than an environmental factor.

3.2.1 Participant Demographics and Eligibility

A total of 18 healthy adults volunteered to participate in this study, n=7 women and n=11 men. Three subjects underwent an additional round of testing for examination of sweat rate variances using the Macroduct®, while a fourth subject was tested four times for sweat rate variance examination. All subjects were de-identified to ensure full anonymity of participants. Ages ranged from 20 to 62 years old. Two subjects indicated a left-handed dominance, while the other 16 indicated a right-handed dominance. Subjects were asked to estimate their average physical activity level, rating themselves in four categories from sedentary (no
physical activity) to very active (>5 workouts per week). Each subject was asked a series of questions to determine eligibility based on predetermined inclusion and exclusion criteria. Exclusion criteria included the subject possessing any of the following: known metal allergies, diabetes, epilepsy, cystic fibrosis, pregnancy, tattoos and/or visible skin irritations on the testing site including eczema, and cardiac or heart problems. Exclusion criteria were determined for the consistency of subject pool.

3.2.2 Nanoduct® Iontophoresis

After the acclimation period, a procedure similar to the recommended procedure provided via Wescor Inc. was utilized for sweat stimulation and electrolyte analysis, including an alcohol-based cleaning procedure at the site of stimulation to remove natural oils. During iontophoresis, a separation of stimulation (red) and return electrode (black) at 1 inch was utilized with the stimulation electrode 3 inches above the subject’s wrist. The pilocarpine-infused hydrogel used for iontophoresis was PILOGEL® (0.5% pilocarpine nitrate) which is also commercially available via Wescor®. For iontophoresis, the Nanoduct® provides a steady current of 0.5 mA, roughly 0.25 mA/cm² for 2.5 minutes. After the end of iontophoresis, the stimulation electrode and PILOGEL® disc were exchanged for a conductivity sensor which estimates the NaCl equivalent concentration (mmol/L) within sweat. Further, the Nanoduct® reports an average NaCl equivalent value after 8 minutes of measurement that represents the average Na⁺ and Cl⁻ concentrations shortly after stimulation, herein reported as the ‘Nanoduct® average’. Upon completion of measurements, all components were removed and the subjects arm thoroughly cleaned.
3.2.3 Macroduct® Iontophoresis

After the acclimation period, a procedure similar to the recommended procedure provided via Wescor® was utilized for sweat stimulation and electrolyte analysis, including an alcohol-based cleaning procedure at the site of stimulation to remove natural oils. During iontophoresis, a separation of stimulation (red) and return electrode (black) at 1 inch was utilized with the stimulation electrode 3 inches above the subject’s wrist. The pilocarpine-infused hydrogel used for iontophoresis was PILOGEL® (0.5% pilocarpine nitrate) which is also commercially available via Wescor®. For iontophoresis, the Macroduct® provides a steady current of 1.5 mA, roughly 0.25 mA/cm² for 5 minutes. After the end of iontophoresis, all electrodes were removed and a collection spiral placed over the stimulation region. This collection spiral relies on the hydrostatic pressure of the sweat glands to fill a spiral tube with a total volume of 85 µL. Further, this collection spiral contains less than 10 nanomoles of water-soluble dye at the entrance to allow visualization of the collection spiral as it fills with sweat. During the collection period, marks were made at regularly scheduled intervals on the spiral to indicate the cumulative amount of sweat generated at each respective time point. Since each centimeter of the spiral corresponds to 2.7 µL of sweat, one can make an accurate estimation of sweat rate. Upon completion of measurements, all components were removed and the subjects arm thoroughly cleaned.

Duplication tests followed an identical procedure, with electrodes being placed on the same sites of the forearms as in the initial Macroduct® test.
3.2.4 Statistical Analysis

Statistical analysis was performed utilizing JMP® statistical discovery software. Tests performed for comparing sweat rates between men versus women and left arm and right arm variations were two-way (factorial) ANOVA for parametric data sets. Maximum sweat rate values are reported as the maximum data set value at the noted time point for each data group ± standard error. Each time point represents the mean of all individual values in that data set. Statistical analyses of the duplication tests for intrapersonal sweat rate comparisons were conducted using one-way ANOVAs for each test of each noted identifier.

3.2.5 Study Approval and Informed Consent

Approval for this study was granted by University of Cincinnati Institutional Review Board (UC IRB). Subjects were thoroughly explained the test procedure and were required to sign an informed consent document before beginning their first appointment test. Subjects who volunteered for a duplication of the Macroduct® test were required to sign an additional informed consent document as if the participant was a new identifier.

3.3 Results

3.3.1 Nanoduct®

As mentioned previously, after 8 minutes of measurement of sweat conductivity the Nanoduct® device reports an average value in mmol/L NaCl equivalents, referred to here as the ‘Nanoduct® average’. Histograms of these data as reported by the device for men vs. women and right vs. left arm are provided in Figure 4. Due to the limited sample pool, statistical significance and normality of these histograms are difficult to interpret. Specifically, in Figure 4(a) the Nanoduct® average for men and women appears to be centered at 35-55 mM NaCl
equivalents. In Figure 4(b) the Nanoduct® average for the left arm indicates a right skewed distribution with a peak around 35-45 mM NaCl equivalents.

**Figure 4** Binned histogram of the Nanoduct® average values, reported 8 minutes after sweat conductivity measurements commenced. (a) Interpersonal men versus women’s estimated NaCl concentrations are displayed. In this data set, n=9 men and n=7 women are displayed. (b) Intrapersonal variations of right arm versus left arm NaCl concentrations are displayed. In this data set, n=16 with 2 subjects excluded as outlying data.

### 3.3.2 Macroduct®

The first four minutes of all sweat rate measurements display a delay in which the sweat collected in the concave portion of the spiral base in order to begin pushing up through the spiral tubing. The peak sweat rate occurred between 8 and 10 minutes for all subjects. Men’s sweat rates peaked at 9 minutes at a rate of approximately 5.2±0.3 µL/minute with a consistent decreasing trend thereafter, where women’s average sweat rates showed a relative plateau from 7-17 minutes at around 2.8±0.2 µL/minute, with the first significant decrease at 20 minutes (p<0.05 for men versus women) (Figure 5(a)). Intrapersonal examinations of sweat rates (right arm versus left arm) displayed a nearly identical overlap of values, peaking at 9 minutes with a continuous decrease in sweat rates (Figure 5(b)). In both comparative groups not all identifiers are present in values after 25 minutes due to a complete filling of sweat rate spiral, which may be related to the final increase in sweat rates.
Macroduct® sweat rate tests. (a) Sweat rates are shown for men versus women with men’s sweat rates peaking at 5.1±0.2 µL/min at 9 minutes, while women’s sweat rates remained statistically constant at 2.8±0.2 µL/min from 5-20 minutes. (b) Sweat rates are shown for right versus left arm. Both right and left arms display nearly identical values for the duration of the test.

3.3.3 Duplication Tests

Four identifiers took place in duplication tests using the same Macroduct® procedure in order to examine variations in sweat rates after multiple dosings. For each identifier, the 8 minute time point was used as a comparative mean value of sweat rate. Identifier 11 showed a week 0 sweat rate of 4.3±0.2 µL/minute and 12 weeks later saw a rate of 3.0±0.4 µL/minute (p<0.05) (Figure 6(a)). Identifier 14 showed a week 0 sweat rate of 3.7±0.2 µL/minute sweat rate and at week 7 displayed a rate of 2.1±0.2 µL/minute (p<0.05) (Figure 6(b)). Identifier 15 showed a week 0 sweat rate of 1.7±0.2 µL/minute and week 12 displayed 1.0±0.2 µL/minute (p<0.05) (Figure 6(c)). Identifier 6 had a total of 4 duplication tests performed at various times to see if a recovery period could be determined. Tests were performed at week 0, 6, and 8, with a final recovery test at week 33. Sweat rates for weeks 0, 6, and 8 displayed values of 2.7±0.3 µL/minute, 2.0±0.2 µL/minute and 1.3±0.1 µL/minute, respectively (p<0.05) (Figure 6(d)). Week 33 shows a near full recovery to a week 0 sweat rate trend; though it should be noted
the sweat rate tapers off more quickly than previous tests and ends closer in the range of the week 6 test.

Figure 6 Duplication tests performed by the Macroduct® device. Values noted are at 8 minutes for each identifier due to this time point being most representative of the entirety of the sweat test values. (a) Week 1 test shows a sweat rate of 4.3±0.2 µL/minute and a week 12 sweat rate of 3.0±0.4 µL/minute (p<0.05). (b) Week 0 displays a sweat rate of 3.7±0.2 µL/minute sweat rate and week 7 displays a sweat rate of 2.1±0.2 µL/minute (p<0.05). (c) Week 0 shows a sweat rate of 1.7±0.2 µL/minute and week 12 displayed 1.0±0.2 µL/minute (p<0.05). (d) Four duplication tests were performed at Week 0, 6, 8 and 33. Sweat rates displayed values of 2.7±0.3 µL/minute, 2.0±0.2 µL/minute, 1.3±0.1 µL/minute, and 2.7±0.3 µL/minute respectively (p<0.05). Week 33 shows a near full recovery to a week 0 sweat rate trend with decline towards the end of test.

3.4 Discussion

Male and female sweat rates as a result of the Macroduct® protocol showed obvious differences, but male and female electrolyte concentration variations as a result of the Nanoduct® protocol did not display a noticeable difference (Figure 4(a)). This result was unexpected as it has been well-documented that Na⁺ and Cl⁻ levels are strongly dependent
upon sweat rate (3, 45). Conversely, left and right arm sweat rates showed little to no
difference, but differences in left and right arm electrolyte concentrations could be noted
(Figure 4(b)). Asymmetry in sweat response in left-handed and right-handed individuals has
been previously reported, as well as quantities of sweat differing per dominant arm (12).
Logically a larger quantity of sweat should indicate higher sweat rates, thereby leading to
differing electrolyte concentrations on opposing limbs. Intrapersonal variances of electrolyte
concentrations should be noted, and we predict they will become statistically significant upon
testing of a larger subject pool. Larger subject pools will also offer greater quantities of left
handed subjects, subsequently offering better evidence towards differences of electrolyte
concentrations based on dominant handedness.

Along with the differences noted in electrolyte concentrations, it can be seen that
sweat rates vary interpersonally between men and women (Figure 5(a)). Humans have an
estimated total eccrine sweat gland count ranging from 2-5 million glands (3, 11, 45). Though
men are known to produce higher amounts of sweat, it is not because their sweat gland count
is higher. Rather, men and women typically have the same number of sweat glands across
their bodies (11). In fact women have a higher sweat gland density, presumably due to having
smaller body frames (11). Thus our data and others show that men produce larger volumes of
sweat than women due to significantly higher sweat rates per gland.

As can be seen with the duplication tests (Figure 6), repeated iontophoresis treatments
lead to lowered sweat rates. This phenomenon is not unseen in the iontophoretic treatments of
hyperhidrosis. Hyperhidrosis is classified as a sweating disorder where the individual
produces physiologically excessive amounts of eccrine sweat, primarily on the hands, face
and feet (53). Iontophoretic treatments to the afflicted area using tap water combined with the
electric current have been proven to show significant reduction in sweating (53, 54).
Hyperhidrosis iontophoretic treatments have examined currents ranging from 15-30 mA, or
whatever is tolerable to the individual (53, 54). Current density of hyperhidrosis iontophoretic treatments are therefore around 0.20 mA/cm², (assuming a 100 cm² surface area; lower current density if the area is larger such as on the feet), which is notably lower than the current density of the two studied Wescor devices (both 0.25 mA/cm²). The physiological mechanism of action to reduce sweating is unknown. One theory behind the phenomenon includes the “plug-theory” where the lumen becomes obstructed through a naturally produced plug due to damaged or released cellular matter and prevents sweat from reaching the surface (55). Noting the sweat rate reductions using commercially available cystic fibrosis tests, we speculate that electric current is causing a disturbance in the sweat gland such as plug formation or damage to the receptors that initiate sweating. It is unclear at this time if pilocarpine itself is contributing to the observed sweat rate reduction.

3.5 Ethical Approval

This study was approved by University of Cincinnati Institutional Review Board (UC IRB).

3.6 Conflict of Interest

The authors declare no conflict of interest. None of the research team members have affiliations with or financial interests in the Wescor company, the manufacturer of the devices tested in this study. Research team member, Jason Heikenfeld, does have a financial interest in Eccrine Systems, Inc. ® which is involved in development of sweat sensors but has no affiliation with Wescor Inc.
4 Exploratory Studies

4.1 Iontophoretic Sudomotor Axon Reflex Patch

Another form of iontophoretic testing is the Quantitative Sudomotor Axon Reflex Test (QSART). This test is designed to evaluate autonomic nerves which control sweating and are connected via a neural network underneath the surface of the skin (56). It is typically used to diagnose nervous system disorders and some pain disorders (56). Stimulation is done with a natural chemical, acetylcholine, placed on the iontophoresis electrodes (56). Upon commencement of iontophoresis sweat glands are activated both directly under the delivery site and also nearby, because of the sudomotor neural network connections. Sweat secretion can then occur up to millimeter distances away from the directly stimulated iontophoretic site.

Currently a patch developed at the University of Cincinnati’s College of Engineering and Applied Sciences is being tested using the QSART test. In the newly developed patch, sudomotor axon reflexes are activated through iontophoretic stimulation using the ActivaDose iontophoresis device at currents similar to or lower than commercially available Wescor Macroduct and Nanoduct devices.

The ActivaDose can provide a current level of as little as 0.1 mA. The ActivaDose automatically calculates the amount of time to run based on the researchers’ inputs of current and desired dose of electrical charge. For example, a current on the ActivaDose set to 0.2 mA for a duration of 2.5 minutes yields a total dose delivered of 30 mC. The total current density in all studies is set to remain at or below 0.25 mA/cm$^2$, which is comparable to the commercial neonatal Nanoduct current density of 0.26 mA/cm$^2$.

The drug presently under consideration for induction of sweat is carbachol within a gel. The gel composition is similar to those used in cystic fibrosis testing. Methacholine (yet another muscarinic agonist) will also be considered as a stimulating agent. These muscarinic
agonists (methacholine and carbachol) possess varying levels of nicotinic activity, a requirement for the activation of sudomotor axon reflexes (52). Sweat glands are difficult to observe with the naked eye but their detection is desired for physiological response studies. For visual detection this patch has an open viewing window containing bromophenol blue along with a thinner of OS-20 produced by Dow Corning cosmetic-grade silicone (PDMS), such as those provided by Clearco Products Inc., to display activated sweat glands.

All components of the patch are constructed with nonhazardous materials replicating components of standard iontophoretic stimulation devices (Figure 6). The full UC IRB approved protocol can be found in Appendix B.

Figure 7 Schematic of QSART iontophoretic patch developed at University of Cincinnati. Displayed are five sensing electrodes surrounded by a stimulation electrode which provides the chemical, sweat stimulant.
The work outlined here will confirm the efficacy of this novel on-skin patch for sweat stimulation and sensing of electrolytes. This study will also validate the efficiency of sudomotor axon reflex sweating for future sweat sensing technologies, with the aim of eliminating one or more of the limitations associated with sweat sensing.

One limitation potentially overcome by this patch is contamination of the sweat sample by sweat stimulation drugs. The sudomotor axon reflex response allows for the sweat stimulating drug to be delivered directly in one location, but allows for nearby sweat glands to activate up to a few millimeters away. The activated sweat glands distant from the stimulation site will not have contact with the sweat stimulation drug, and therefore will produce virgin sweat.

A second limitation overcome is the evaporation of the sweat sample leading to imprecise measurements of sweat analyte concentrations. The design of this patch provides protecting plastic covering over the stimulated sudomotor activated sweat glands. The plastic additionally covers the sensing electrodes, allowing for the true concentrations of sweat at non-skewed volumes to be detected. Simultaneous sweat response and sensing of electrolyte values allows for trends in active sweat to be determined, leading to confirmation of sweat rate and concentration comparisons.

The testing of this patch is on-going. Results will be available for public disclosure upon completion of recruitment and de-identification of all consenting identifiers.

4.2 Exercise Patch

A second patch, also developed at University of Cincinnati’s College of Engineering and Applied Sciences, moves away from the standard iontophoretic stimulation of sweat glands and focuses on the body’s natural sweat response through exercise. This patch is designed as a Band-Aid-like adhesive that incorporates Bluetooth technology for transmission of sweat response. Sweat responses detectable are sweat rate, sweat electrolyte concentrations
including $\text{Na}^+$, $\text{K}^+$, and $\text{Cl}^-$, and skin surface temperature. The full UC IRB approved protocol can be found in Appendix C. Testing of this sweat patch has yet to commence although protocol approval has been obtained.
5 Conclusions and Recommendations

Sweat is growing in popularity in the biomedical community as a potential alternative to current invasive diagnostic techniques due to its ease of access and analyte concentration correlations to other body fluids such as blood, urine and saliva. Sweat sampling, however, has numerous limitations which include: lack of proper sampling devices and limited analyte detection capabilities; sample evaporation; difficulty in producing and collecting adequate sample sizes for analysis; contamination of samples from epithelial matter and/or sweat stimulation drugs; and large variability in sweat analyte concentrations from person to person as well as regionally on a body (57-59).

Commercially available devices give researchers a starting ground from which to build. Novel sweat sensing patches implementing components of commercially available technologies are currently under development to further increase sweat’s value as a biofluid. However for these technologies to be developed into consumer-friendly, efficient, wearable devices a few critical weaknesses of current commercially available sweat stimulation and sensing devices must be overcome.

First, future sensor development must include stimulation and sensing into one simultaneous step. Though the Nanoduct® device’s digital screen displays an initial sweat rate, sweat rate changes post stimulation cannot be detected with the Nanoduct® device. Conversely the Macroduct® sweat test does not offer immediate electrolyte concentrations, and also does not provide a continuous read out of sweat rate. At present sweat rate values must be obtained manually using volumetric estimation at specified time points as described in the human subjects study in Chapter 3. Correlations between sweat rate and certain analyte concentrations may be found in many literature sources (45). However, development of a sensor which can offer instantaneous feedback of the analyte concentrations and corresponding sweat rate is highly attractive.
Second, future device design must incorporate a lowered iontophoretic current in order to prevent the decrease of sweat rates after multiple tests on the same site as demonstrated in Chapter 3. Future technologies pose the potential for continuous biomonitoring, but in order to do so must not cause depletion in sweat rate of the consumer. At best, such a situation would require increased drug dosages to maintain an appropriate sweat rate. Lower current may also prevent discomfort and promote compliance for use.

Finally, sensor development should incorporate additional analyte sensing capabilities. The Nanoduct® device outputs a value for mM NaCl equivalents, and the Macroduct® stimulated sweat is analyzed via laboratory methods for chloride ion concentrations. In order for future sensors to provide means of detection for conditions other than cystic fibrosis, human performance and other biomonitoring sensors with increased analyte capacity must be developed.

At no point during sweat sensor development should human subjects testing be overlooked. The certainty of intra- and interpersonal variations of sweat rates and concentrations dictates that any development should and must be tested on human subjects in order to confirm efficacy. Intra- and interpersonal variabilities in the numerous biomarkers in sweat precludes requires a thorough study of these factors that should not be postponed. While human subjects testing is viewed as tedious it must be remembered that the safety of human subjects is the number one most important priority. A priority requirement for effective sensors and sweat stimulation devices is a minimal irritation during and after stimulation. This will lead to higher participant compliance as well as lower risk, making human subjects study approvals and execution that much simpler.

Devices currently under development address many of the previously mentioned shortcomings dealing with sweat as a diagnostic biofluid. These shortcomings lead to a level of skepticism in some circles. However through additional human subjects testing and
mindful elimination of device limitations sweat sensing may achieve a position of strength in the world of minimally invasive biomarker analysis.
Bibliography

Appendix A: Testing the Microfluidics of On-Skin Technologies

UNIVERSITY OF CINCINNATI
INSTITUTIONAL REVIEW BOARD – SOCIAL AND BEHAVIORAL SCIENCES (IRB-S)
PROTOCOL

TITLE:
Testing the Microfluidics of On-Skin Technologies

Trudy Gaillard (gaillaty@ucmail.uc.edu)
Gerald Kasting (kastingb@ucmail.uc.edu)
Jason Heikenfeld (heikenjc@ucmail.uc.edu)
Eliza Wilder (wilderec@mail.uc.edu)
Terri LaCount (lacounj@aol.com)
Zachary Sonner (sonnerzc@mail.uc.edu)

PURPOSE of the research project AND GENERAL INFORMATION:

PURPOSE
The purpose of this research study is to recreate the Food and Drug Administration (FDA) approved Cystic Fibrosis test using the Wescor Nanoduct device and the Wescor Macroduct device. Both devices stimulate sweat by passing a drug known as pilocarpine through skin through a small electrical current. These tests will allow us to measure sweat electrolyte concentrations versus sweat rate in individuals as well as visibly monitoring sweat secretion.

BACKGROUND
Prior research
Wescor Nanoduct and Macroduct units have been used in diagnosis of infantile Cystic Fibrosis for many years through induction of sweat and simultaneous detection of electrolyte levels. The both devices stimulate sweat through low current iontophoresis of pilocarpine drug through skin. The Nanoduct unit determines sweat electrolyte concentrations in NaCl equivalents, whereas the Macroduct unit requires sweat to be collected in coiled spirals and analyzed manually. Cystic Fibrosis patients have been shown to display high levels of chloride ions in their sweat due to a malfunctioning Cystic Fibrosis Transmembrane Regulator in the gland walls. The both devices have small electrodes for neonatal patient testing and low amperage. These devices are FDA approved and are standard procedures used to diagnose infants with Cystic Fibrosis.

Significance
This project will be part of a validation study for an on-going research project involving members of the University of Cincinnati (College of Engineering and Applied Sciences, College of Pharmacy, and College of Nursing). The project goal is to detect levels of various molecules in sweat and compare them to sweat rate. The trials will confirm that certain electrolyte concentrations should be found versus varying sweat rates in healthy adults. The Nanoduct unit will provide an automated calculation of sweat rate, where the Macroduct unit will allow for manual calculation of sweat rate.

FUNDING
Sponsor's name and type
The sponsor of this research study is G.B. Kasting who has generously offered initial start-up funding from his personal faculty account for the purposes of this project.
Sponsor's role
G.B. Kasting is a Co-PI on this research study.

Location of funds
University of Cincinnati College of Pharmacy

Status of funding
Funds are currently available to use at his discretion. We will be seeking funding for future clinical aspects of this project through Cincinnati Center for Clinical and Translational Science and Training (CCTST).

FACILITIES
Research will be conducted at the University of Cincinnati in a lab within the James L. Winkle College of Pharmacy.

DURATION OF STUDY
This research study will be completed within 4 months of IRB approval.

RESEARCH TEAM
Research team and time commitment

<table>
<thead>
<tr>
<th>Job Title / Responsibility</th>
<th>Time Commitment</th>
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<tbody>
<tr>
<td>PI / Monitor on-going tests, store data, assist with analysis</td>
<td>4 months</td>
</tr>
<tr>
<td>Co-PI (Faculty Advisor) / Assist PI with any supplemental info</td>
<td>4 months</td>
</tr>
<tr>
<td>Co-PI (Faculty Advisor) / Assist PI with an additional testing</td>
<td>4 months</td>
</tr>
<tr>
<td>Co-Investigator / Conduct tests, record results, analyze data</td>
<td>4 months</td>
</tr>
<tr>
<td>Co-Investigator / Conduct tests, record results, analyze data</td>
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</tr>
<tr>
<td>Co-Investigator / Conduct tests, record results, analyze data</td>
<td>4 months</td>
</tr>
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Training team members in research ethics
All researchers have completed the CITI training in ethics of human research.

Training team members in research activities
Training
A step-by-step detailed set of instructions will be given to each researcher. Each researcher will then need to have thorough understanding of the procedure. The PI and Co-Investigators will run mock trials of the protocol to ensure a definitive understand of the protocol.

Verification
Research team members will be required to have a copy of the protocol with them at all times during testing. The PI and Co-PIs will be responsible for ensuring all compliance with the testing procedures by Co-Investigators.
PARTICIPANTS:

RECRUITMENT
Study participants will be recruited via word-of-mouth and flyers located around campus.

Number of participants
Minimum and maximum number of participants
A minimum of 10 participants will be sufficient for analysis. Conversely a maximum of 25 participants will participate. The 25 subjects will be the same for both sets of testing.

Rationale
Based on a pre-study power calculation assuming 15% coefficient of variation, 15 subjects will allow the detection of a 25% difference in the means with an 80% power assuming normal distribution at a two-sided 5% nominal significance level. This is a validation study so we are allotting a sufficient amount of subjects to participate.

Inclusion and exclusion criteria
Subjects are included in this study if they are above the age of 18 years old and are able to give consent. Subjects will be excluded if they possess eczema or additional conditions on forearm(s), have known metal allergies, have tattoo(s) on forearm(s), have preexisting cardiac/heart conditions, have epilepsy, have Cystic Fibrosis, and/or are currently pregnant. Conditions such as pregnancy will not cause any harm on the mother or the child if the woman is pregnant. Indicated previously in a study by Jack Lieberman, “it is know that estrogens and progesterone may cause sodium, chloride, and water retention…” Women involved in this study will be asked the date of their last menstrual cycle to confirm they are not pregnant. The inclusion and exclusion criteria are selected by our research team in order to perform tests on as consistent of a subject pool as possible with no physiologically elevated levels of electrolytes.

Vulnerable participants
Vulnerable participants will be excluded from the study.

Vulnerability
No vulnerable subject is involved.

Rationale
Not applicable.

Confirmation
Driver’s License or other form of identification will be used to confirm a participant is 18 years of age or older. The area of testing (forearms) will be thoroughly examined before application of device. Verbal confirmation will be given by the subject to validate the subject does not possess one of the above mentioned conditions which would exclude him/her from the study.
Risks and discomforts from participating
Type and level of risk or discomfort

<table>
<thead>
<tr>
<th>Risk or Discomfort</th>
<th>Level</th>
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<tr>
<td>Redness (erythema) or irritation associated with chemical stimulus after removal of electrodes</td>
<td>Minimal</td>
</tr>
<tr>
<td>Redness (erythema) or irritation associated with electrical stimulus after removal of electrodes</td>
<td>Minimal</td>
</tr>
<tr>
<td>Tingling or prickling sensation during iontophoretic delivery</td>
<td>Minimal</td>
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</tbody>
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Safety monitoring plan
Research participants will be constantly monitored during testing. If any irritation above minimal occurs the test will be terminated immediately. Research staff will review data and efficacy of data.

Reporting
Notification of PI
The research staff will be present at time of testing and will be notified via the research team immediately upon indication of adverse irritation.

Notification of IRB
The IRB will be notified immediately upon termination of test via explanatory email or phone call.

Other notification
Not applicable.

Available resources
After sweat collection has reached completion patients will be given a topical dosage of hydrocortisone cream or warm compresses if irritation occurs.

Direct benefits to the participant
There are no direct benefits to the participant.

Recruitment activities
Recruitment materials
List: Flyer, Word-of-mouth

Personnel
The research staff will be responsible for posting flyers and speaking with potential subjects.

Recruitment activities
The research staff will hang flyers across the east and west campuses of the University of Cincinnati.

Participant response
Participants will contact a member of the research staff by email to arrange a time for the testing to take place.
CONSENT PROCESS
Presenting information to potential participants
All potential participants will be expected to understand and comprehend sufficient English if registered in University of Cincinnati classes.
A consistent email template will be used for each member of the research staff to send to potential participants when they contact a member of the research team.

Answering questions from potential participants
Participants will be given the opportunity to ask questions via email or before the test begins.

Indicating consent
Participants will be required to sign and date an Informed Consent Document (ICD) given at the time of testing.

Legally authorized representative (LAR) for minors or cognitively impaired participants
Not applicable.

Verification of LAR for cognitively impaired participants
Not applicable.

Avoiding coercion
Not applicable.

Recruitment incentives
A $10 gift card to a local restaurant will be distributed at time of completion of each test. If test is terminated early the participant will be equally compensated.

CONSENT DOCUMENTS (ICDs)
List: Testing the Microfluidics of On-Skin Technologies ICD

RESEARCH-RELATED ACTIVITY:
SECONDARY ANALYSIS of an EXISTING DATASET
Not applicable.

REVIEW OF RECORDS that were collected for NON-RESEARCH PURPOSES
Not applicable

RESEARCH ACTIVITIES
Privacy of participation
All data is de-identified and used for validation purposes only.

Confidentiality of data
Names will not be correlated with any other identifiers or data results. Data collected will consist of age, gender, physical activity, sweat electrolyte concentrations, and sweat rates. All pieces of information regarding the participant and data collected during the test will be confidentially kept in the office of the research staff on a password-protected computer.
Activities and duration

*Nanoduct*

The device will be calibrated prior to experimentation. All jewelry will be removed. The following procedure will be performed on both arms to ensure consistency in results per subject. The left arm will be recorded first; after completion of the test on the left arm, the right arm will have the same procedure performed on it.

- Acclimate subject at 20 deg. C and ~20% relative humidity (RH) for 30 minutes prior to test.
- Remove Pilogel disks from refrigerator 15 minutes prior to use.
- Swab a large area of skin on inside forearm with alcohol. Follow that with deionized (DI) water and KimWipes.
- Place the black holder on portion of arm closer to face (i.e., about 2/3 way up arm from wrist), and secure with band; then place red holder on the arm in a similar manner approximately 1.5-2” away and nearer to the wrist. Do not place on wrist or tendon area.
- Place 1 drop of DI water into each electrode surface (disk holder), then place Pilogel disk onto each by touching the sides of disks but not flat surfaces; do not use disk if cracked or edges are chipped/cracked.
- Add 1 drop of DI water in middle of holder directly onto skin.
- Place electrodes containing disks into appropriate holders and twist lightly downwards to lock into place. Arrow indicator should be aligned (counterclockwise) and electrode should lock into place.

After placement into holders, ensure electrode wires are securely attached to patient’s arm.

- Turn device on.
- Connect cable to socket of inducer analyzer.
- “Iontophoresis” should be selected. Press enter. Display will show increase in current. Once current has reached full 0.5 mA, this will be maintained for 2 minutes. Patient should remain relatively still during the 2 minute process. Current will then decrease to 0 and the analyzer will beep.
- After the device has concluded induction, the display will show “Iontophoresis Complete”. Press enter to return to main menu.

- After completion of iontophoresis, remove red electrode by twisting, but leave both holders and black cathodic electrode in place for ground contact for sweat rate determination.
Macroduct

All jewelry will be removed. The following procedure will be performed on both arms to ensure consistency in results per subject. The left arm will be recorded first; after completion of the test on the left arm, the right arm will have the same procedure performed on it.

- Remove Pilogel disks from refrigerator 15 minutes prior to use.
- Swab a large area of skin on inside forearm with alcohol, following with deionized (DI) water.
- Place 1 drop of DI water into each electrode surface, then place Pilogel disk onto each electrode. Disks will not be used if cracked or chipped around edges.
- Place 1 drop of DI water onto each disk after insertion into electrodes.
- Place the black electrode onto upper forearm, disk side down (approximately 2/3 way up arm from wrist). Secure with black electrode Velcro-band.
- Place the red electrode on mid-lower forearm, disk side down (approximately 2” from wrist). Secure with red electrode Velcro-band.
- Turn device on. Timer will automatically start to for induction. Device will beep and automatically shut off after 5 minute sweat stimulation. Subject should remain moderately still.
- During stimulation interval remove Macroduct collection spirals from packaging, making sure not to touch back of spiral. Slide securing straps (soft side up) through side slots.
- Remove electrodes at the end of 5 minute induction interval.
- Clean sweat area with DI water and KimWipes. Discard used disks in trash.
- Slide the Macroduct collection spiral onto arm and secure with bands in the location of the red electrode. Firmly pull straps into place. Note to subject that he/she should not clench fist during collection. Collect sweat for ~30 minutes or until no sweat is visibly moving into spiral.
- Once sweat is finished being collected, the collection spiral can be removed from arm. Collection spiral can be sealed off to prevent seat leakage.
- Wipe arm with DI water and KimWipe.

Data collection tools
List: Testing the Microfluidics of On-Skin Technologies Data Sheet

Payments to participants: reimbursement of expenses or payment for time and effort
A $10 gift card to a restaurant will be given to participants after each test.
DATA ANALYSIS:

Nanoduct
- Analysis of sweat is recorded in the Nanoduct device’s internal computer.
- Remove conductivity sensor from bag, being careful not to touch conical surface of sensor.
- Attach sensor to sensor connector on cable.
- Wash stimulated skin area in red area with gauze pads soaked in DI water, and dry with dry gauze pads, being careful not to disturb red holder.
- Confirm induction analysis module is on. Immediately after sensor is in holder, select “Sweat Test” and choose to either display reading during test or read printout later.
- With sensor in place display will indicate sweat contact with electrode within minutes. Initial rate is shown in g/m²/min and can range from 0.5 units to 10 units. After another 2-6 minutes, conductivity display shows continuous data, and after 3 minutes average begins to be taken. The mean conductivity is displayed after 5 minutes.
- Once test is complete the analyzer beeps 3 times. Ensure no errors are present. Record mean conductivity result.
- Press enter to return to main menu.
- Begin disassembly of device:
  - Disconnect cable from sensor.
  - Remove sensor from holder.
  - Discard used sensor.
  - Disconnect cable plug from the socket of sweat analysis module.
  - Turn off power.
  - Remove black electrode from holder.
  - Black and red holder gel discs may then be removed and discarded.
  - Remove holders from patient.
  - Gently wash and dry skin with DI water and gauze pads.
  - Wash and dry electrodes with alcohol wipes. Clean straps and holders with gauze pads damped with bleach.

*Mean sweat conductivity results is in mmol/L; normal expected results are 0-60mmol/L (eq. in NaCl).

Macroduct
As sweat is being collected through the collection spirals, small tick marks will be made on collection spiral at predetermined time intervals for later calculation of sweat rate versus the volume at the given times.
Appendix B: Testing of a Novel Iontophoretic Sweat Stimulation Patch

Principle Investigator: Trudy Gaillard

Title: Testing of a Novel Iontophoretic Sweat Stimulation Patch

Specific Aims
The purpose of this research study is to validate the efficacy of a novel on-skin patch through iontophoretic stimulation. The patch will then show a real-time activation of sudomotor nerves by causing nearby sweat glands to activate. The validation of activation of sudomotor nerves in sweat’s physiological response will give way to future sweat sensing technologies. Sweat gland innervation will be monitored via visual and electrical methods.

Background and Significance
Sweat is a biofluid which can easily be accessed through non-invasive techniques. Sweat possesses many biomarkers that can be used for monitoring stress levels and also contains certain electrolytes which are used for diagnostic purposes. The Cystic Fibrosis (CF) sweat test is the gold standard, routine procedure performed on infants and young children to determine levels of chloride ions in sweat. Sweat is generated with FDA approved devices, Wescor Macroduct and Wescor Nanoduct, via iontophoretic delivery of a sweat stimulant called pilocarpine in gel form. These devices drive a small current from one electrode to another across the skin while simultaneously delivering a small dose of a sweat gland stimulating drug called pilocarpine. Increased levels of chloride ions in the patient’s collected sweat indicate a mutation in the Cystic Fibrosis Transmembrane Regulator (CFTR) and, inevitably, Cystic Fibrosis. Another form of iontophoretic testing is the Quantitative Sudomotor Axon Reflex Test (QSART). This test is designed to evaluate autonomic nerves which control sweating and are connected via a neural network underneath the surface of the skin. This test is typically used to diagnose nervous system disorders and some pain disorders. Typical stimulation is done with a natural chemical, acetylcholine.

The work proposed here will confirm the efficacy of this novel on-skin patch for sweat stimulation and sensing of electrolytes. This study will also validate the efficiency of sudomotor axon reflex sweating for future sweat sensing technologies. Iontophoretic stimulation of sweat will allow monitoring of skin impedance and sweat sodium levels thereby helping guide future developments of similar sweat patches for biomedical and consumer applications.

Preliminary Studies
A current IRB approved study entitled Testing the Microfluidics of On-Skin Technologies, led by Principle Investigator Trudy Gaillard, is working towards recreation of the Wescor Cystic Fibrosis test. The PI and her research team are performing both the Wescor Macroduct test and the Wescor Nanoduct test for validation of sweat rates and electrolyte concentrations using pilocarpine iontophoresis.

Investigator Experience
Trudy Gaillard currently works at the University of Cincinnati’s College of Nursing as an Assistant Professor. She most recently previously worked at The Ohio State University in the Division of Endocrinology, Diabetes, and Metabolism as an Assistant Professor of
Experimental Design and Methods

Methods and Procedures

In the newly developed patch sudomotor axon reflexes will be activated through iontophoretic stimulation using the ActivaDose iontophoresis device at currents similar to or lower than commercially available Wescor Macroduct and Nanoduct devices. ActivaDose is used in cranial iontophoresis. The ActivaDose can provide a current level of as little as 0.1 mA. The device will never be set to higher than 0.5 mA. The ActivaDose automatically detects the amount of time to run based on the researchers’ inputs of current and desired dosage. For example, a current on the ActivaDose set to 0.2 mA for a duration of 2.5 minutes offering a total dosage delivered to be 30 mC. The total current density in all studies will remain at or below 0.25 mA/cm², which is comparable to the commercial neonatal Nanoduct current density of 0.26 mA/cm². This setting selected for the initial patient; however if the patient exudes sweating at a current level of 0.2 mA, the following patient may be selected to have a current of 0.1 mA delivered from the ActivaDose for measuring sweat response at extremely low levels of stimulation. If the patient does not exude a sweat response, the following patient may be selected to have a current of 0.3, 0.4, or 0.5 mA delivered. The ActivaDose will never be set higher than 0.5 mA (or exceed 0.25 mA/cm²), being that is the current produced by the commercially available Nanoduct. Members of the research team will be specially trained in the use of the ActivaDose and its controls. The device does not store previous inputs in a centralized computer.

The drug to be delivered for induction of sweat will be carbachol within a gel, similar to the gels used in the previously mentioned Cystic Fibrosis testing. Carbachol is typically used in eye drops to treat glaucoma. Additionally a previous study has shown to use carbachol in iontophoretic stimulation of sweat mimicking the QSART test where the carbachol was used in replacement of acetylcholine (Reidl 2001). The use of methacholine (yet another muscarinic agonist) will be available for use in comparison purposes of efficacy of chemical stimulus for the patches. Both mentioned muscarinic agonists (methacholine, and carbachol) possess varying levels of nicotinic activity which is a requirement for the activation of sudomotor axon reflexes. Sweat gland detection is difficult for the naked eye to visual but is desired for physiological response studies. Bromophenol blue (BMP BLUE) has been previously used in sweat gland visual detection in a solution of silicone oil (PDMS base) (Tashiro, 1960). For visual detection our patch will have an open viewing window containing BMP and a cosmetic-grade silicone (PDMS), such as those provided by Clearco, to display activated sweat glands.

For reader familiarity a general description and pictures of the patch is attached in document SAR Device Prototype. All components of the patch are constructed with nonhazardous materials replicating components of standard iontophoretic stimulation devices. Final device design is subject to alteration to reduce subject risk and/or improve data quality. Carbachol will be the initial drug used in iontophoresis, however if carbachol proves to be ineffective as a stimulus methacholine will be available for a replacement stimulus.
Procedure:
1) Clean patch testing area (randomized selection of forearm) with rubbing alcohol followed by deionized water to rid the skin of excess epithelial matter and allowed to air dry.
2) Current on the ActivaDose will be set to 0.2 mA for a duration of 2.5 minutes offering a total dosage delivered to be 30 mC. The total current density will be at or less than 0.25 mA/cm², which is comparable to the commercial neonatal Nanoduct current density of 0.26 mA/cm². Drug-containing gel will be previously cast or formulated and placed above the open stimulation/return electrode regions to create a secure skin contact surface.
3) Ensure strong adhesive contact between the patch and the skin while simultaneously providing slight compression force to ensure strong gel-skin contact.
4) Device will be turned on and iontophoresis of drug stimulation will commence.
5) A small drop of pre-made silicone solution will be placed on skin in a small open viewing window of the patch.
6) During and after iontophoresis, the subject will remain sitting still while pictures of the viewing window are taken every minute for 20 minutes. Similarly, the sensing electrodes will be monitored and data recorded during this time.
7) After a 20 minute time interval of sweating the patch will be removed from the subject’s arm. Area will be initially wiped with a dry towel to remove oil and BMP BLUE mixture. Then the area will be washed with soap and water to remove any remaining residue and staining. Hydrocortisone cream will be given to the subject for application if any irritation occurs.

Data Analysis and Data Monitoring
Data will be de-identifiable and reported in aggregate form at the completion of all tests. Data will be reported in the form of charts, graphs, or tables once all tests are completed. Photographs of patch viewing windows will also be analyzed and de-identified.

Data Storage and Confidentiality
Names will not be correlated with any other identifiers or data results. All subjects will be de-identified. Data collected for the study will consist of age, gender, physical activity, and sweat patch results which include skin impedance through sweat rate and sweat sodium concentration that will be detected through sodium ionophore sensors. Some of this data will be stored electronically in table format. Additionally photographs of the open window of the patch will be taken during sweating to show live action of the autonomic nerves producing sweat. Any photographs taken will be strictly of the test site on the subject to ensure there is complete anonymity of the participant. All pieces of information regarding the participant and data collected during the test will be confidentially kept in the office of the research staff on a password-protected computer and hard copies kept in a locked filing cabinet. The computer password will only be given to the research staff as well as the key to the locked filing cabinet.

Setting
The iontophoretic stimulation will be conducted at the University of Cincinnati’s College of Engineering and Applied Sciences.

Laboratory methods and facilities
Not applicable.
Estimated Period of Time to Complete the Study

Each test will take approximately one hour of the subject’s time. The full duration of the study will take 6 months from initial subject testing to final analysis and write-ups of sweat patch efficacy.

Human Subjects

Research team members will recruit age and sex match males and females above the age of 18 years old who are able to give informed consent. Study participants will be excluded if they are currently pregnant, have preexisting cardiac/heart conditions, have epilepsy, have Cystic Fibrosis, possess tattoos, eczema or additional skin conditions on patch application site (forearm). In addition we will exclude subjects with hypertension, diabetes, or known metal allergies. There are no gender biases in this study, however, as close to an equal amount of men and women are desired for this study. A valid driver’s license or other form of identification that clearly displays the subject’s date of birth will be used to confirm age requirements before participation in the study. The area of patch application will be thoroughly examined before adherence of device. Verbal confirmation will be given by the subject to validate the subject does not possess one of the above mentioned conditions, to the best of his or her knowledge, which would exclude him/her from the study. No vulnerable subjects will be included in this study. Up to 20 patients will be enrolled in this study at this site. Approximately 20 patients at 1 site in the U.S. will be enrolled in the study. The subjects will predominantly be recruited from the student population of the University of Cincinnati. No students of the PI or the research staff will be involved in this study. The research team will be responsible for conveying the research study information to potential subjects via word of mouth. The potential subjects will then be given the research staff’s e-mail in order to schedule a testing time if the subject does decide to become a participant.

Risk/Benefit Assessment

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<thead>
<tr>
<th>Risk or Discomfort</th>
<th>Level</th>
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<tbody>
<tr>
<td>Redness (erythema) or irritation associated with chemical stimulus after removal of electrodes</td>
<td>Minimal</td>
</tr>
<tr>
<td>Redness (erythema) or irritation associated with electrical stimulus after removal of electrodes</td>
<td>Minimal</td>
</tr>
<tr>
<td>Tingling or prickling sensation during iontophoretic delivery</td>
<td>Minimal</td>
</tr>
<tr>
<td>Irritation or skin staining associated with silicone solution used in visual detection of sweat</td>
<td>Minimal</td>
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</table>

Subjects will be monitored at all times during the testing procedure. If any irritation occurs above minimal at any point during the testing procedure or the subject is uncomfortable and does not feel he/she would like to continue the test, the test will be terminated immediately without question. A warm compress and hydrocortisone cream will be applied to the skin to prevent further irritation. The site will be monitored for an additional 15 minutes after indication of irritation to assure it does not spread. To minimize any adverse effect associated with the application of the sweat sensor the research team will carefully examine the skin of the application site and thoroughly clean the skin with a topical cleanser.

The PI will be present or on-call at all times during testing. If a subject begins to experience discomfort the PI will be immediately notified and the study team will document the site of irritation and terminate the study.
Payment
The subjects will be awarded with a $10 gift card to a local restaurant at the time of each test. If the subject decides to terminate the test early for any reason he/she will still be awarded the gift card.

Subject Costs
There are no costs to the subjects.

Consent Form
Testing of a Novel Iontophoretic Sweat Stimulation Patch_ICD
Appendix C: Testing of Novel On-Skin Sweat Patch through Metabolic Sweat Induction

Principle Investigator: Trudy Gaillard

Title: Testing of Novel On-Skin Sweat Patch Through Metabolic Sweat Induction

Specific Aims
The purpose of this research study is to validate the efficacy of a novel on-skin patch which will be able to wirelessly transmit skin surface temperature, impedance of skin, and concentrations of electrolytes found in sweat such as sodium, potassium, and chloride.

Background and Significance
Industry has seen attempts made at wearable devices that make electrical or electrochemical measurements for performance monitoring, physiological event detection, and medical condition testing. Most of these devices are either some type of jewelry accessory (i.e. watch, bracelet, etc.) that must be held in contact with the body through clothing or other obtrusive means (i.e. compression bandages/wraps). Few of these devices have been adopted by users because they are too bulky, uncomfortable, noticeable, or hard to use. Even fewer actively use sweat as the source of the target to be measured. Current sweat analyte measurement methods typically require laboratory conditions, expensive and non-portable equipment, and/or are time-consuming and expensive. The innovations presented here combine the novel science of sweat sensing with cutting-edge, low-profile, unobtrusive, wearable electronics to provide a truly mobile platform that enables advances in understanding the roles and applications of biomarkers in sweat.

Previously, we reported an adhesive RFI sensor bandage (patch), which can be made completely intimate with human skin, and therefore minimize dead volumes of sweat for improved chronological monitoring. A commercial RFID chip was adapted with minimum components to allow potentiometric sensing of electrolytes in sweat, and also a reading of surface temperature. Together, electrolytes and temperature could be of use for hydration and heat-stress monitoring.

Sweat is one example of a non-invasively accessed biofluid with potential advantages in measurement of inflammatory biomarkers compared to saliva and potentially superior time-resolved readings of biomarker concentrations compared to saliva and urine. The mineral based elements of sweat have been documented as a product of heat stress from prolonged exertion, and electrolyte concentrations in sweat have found diagnostic use. The gold standard of sweat testing is the diagnosis of cystic fibrosis (CF) by pediatricians to confirm a clinical presentation. Despite advancements in understanding the genetics of this disease, the sweat test remains the predominant confirmation method due to high cost of genetic testing and the proliferation of mutations in the CFTR gene. The sweat test for CF analyzes electrolyte concentration, conductivity, and/or osmolality of a sweat sample collected using absorption-based methods such as the Wescor, Inc. Macroduct sweat testing system. Sweat testing and analysis is also deployed as a method of drug abuse detection, deodorant evaluation, and in the study of dehydration in athletes. Rapid detection of biomarkers in a flexible electronic patch requires label-free and real-time transduction. A common approach is to use an ion-selective electrode (ISE) or other ion-sensitive transducer such as those used in chloride measurements of sweat tests for CF. An equally common, but more exotic, technique is to engineer a bio-recognition element (BRE) bonded to a sensor/transducer, capable of sensitively and selectively binding or releasing the biomarker as
concentration changes in solution. Another viable approach is to sense with arrays of semi-
specific, but distinct, transducers and then statistically determine physiological condition.

Biorecognition elements can be based on nucleotides, amino acids or antibodies. However, many of the analytes of interest are simple measurements of pH, inorganic ion concentration, or metabolites such as lactate, phosphate, etc. These are typically much higher in concentration (μM to mM) than traditional ‘biomarkers’ and require neither sample preparation nor added solutes. As such, these measurements can be made with simple ISE arrays and thereby reduce complexity.

The work proposed here is significant to the understanding and characterization of sweat biomarkers as they pertain to both human performance and physiological events, as well the sampling methods and measurement techniques used to acquire these biomarkers. In-situ measurement of sweat biomarkers via wearable and wireless electronic sensing platforms would provide significant advantages to understanding in near real-time the changes in the physio-chemistry of human sweat during activities that preclude more traditional test methods for the same biomarkers. Such advantages allow for significant advances in individual health monitoring, emergency medicine, and preventative measures for athletic and occupational injury.

Preliminary Studies

A current IRB approved study entitled Testing the Microfluidics of On-Skin Technologies, led by Principle Investigator Trudy Gaillard, is working towards recreation of the Wescor Cystic Fibrosis test. The PI and her research team are performing both the Wescor Macroduct test and the Wescor Nanoduct test for validation of sweat rates and electrolyte concentrations using pilocarpine iontophoresis.

Investigator Experience

Trudy Gaillard currently works at the University of Cincinnati’s College of Nursing as an Assistant Professor. She most recently previously worked at The Ohio State University in the Division of Endocrinology, Diabetes, and Metabolism as an Assistant Professor of Medicine. Dr. Gaillard has been involved with many studies and projects working towards the management and research of diabetes and diabetic patients. Research team member, Jason Heikenfeld, has a fiduciary stake in Eccrine Systems LLC. Eccrine Systems does not have a financial or personal role in this study, however they do license the UC technology, a part of which may or may not be tested on human subjects for this study. Full funding does not exist for this study, but Eccrine Systems LLC has indicated a potential interest in future financial backings. However the research team has made sure there is no compromise of study data for this conflict.

Experimental Design and Methods

Methods and Procedures

The test performed will consist of the patient biking on a stationary bike for a period of 20-30 minutes to induce sweating for measurement of the efficacy of novel on-skin patches. The bike is a Monarch Leg Ergometer. It has a Polar Monitor for heart rate interference. Heart rate will be recorded every 5 minutes. Though the subjects will be asked to increase resistance and/or peddling rate in order to simultaneously increase sweat rate, the subject can choose whether or not to do so with no consequence. Increasing sweat flow will allow for optimal detection of patch efficacy, but will not de-validate the results. The active portion’s purpose is to cause the patient to begin sweating and maintain a flow of sweat for a period of time to get a sweat rate profile at low rates and higher rates. Heart rate (bpm) and resistance (workload) will be displayed by the bike’s digital screen. Subjects will be
responsible for adjusting resistance during the test and will be free to increase or decrease resistance whenever desired by subject if discomfort occurs. Based on the participant’s age, if the participant’s heart rate reaches the correlating value on the chart below the test will be immediately aborted.

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<tr>
<th>Age</th>
<th>HR&lt;sub&gt;max&lt;/sub&gt;</th>
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<td>30</td>
<td>185</td>
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</table>

Vitals of the participant will be taken before and after the test and include pulse oximetry, blood pressure, heart rate and body temperature. Trained paramedics will be on standby to assist with vitals and monitoring patient health.

- Clean patch testing area (forearm of preference) with rubbing alcohol followed by deionized water to rid the skin of excess epithelial matter and allowed to air dry.
- Secure adhesive patch to forearm.
- Establish wireless connection between patch and external device during which the device will automatically calibrate.
- Subject will mount bike and adjust seat to a comfortable position and height.
- Subject will begin to peddle at a workload they select as a so-called “flat road” for 5 minutes.
- After 5 minutes the subject will be asked to increase their resistance to a slightly higher work-load and/or peddle at a faster rate. The subject will continue pedalling at this pace for 15 minutes. If the subject does not wish to increase their work-load they are under no obligation to do so.
- After the 15 minute work period, the subject will decrease the workload back to their flat-road for a cool-down period from 5-10 minutes as deemed necessary by the subject.
- Subject will dismount the bike, stretch, and patch will be removed from arm. Subject will be given a cold towel, water, or other post-exercise materials he or she needs.
- Bike will be thoroughly cleaned.

Data Analysis and Data Monitoring
Data will be de-identifiable and reported in aggregate form at the completion of all tests. Data will be reported in the form of charts, graphs, or tables once all tests are completed.

Data Storage and Confidentiality
Each participant will be given a de-identifiable study number. Names will not be correlated with any other identifiers or data results. Data collected for the study will consist of age, gender, physical activity, heart rates during test, resistance of bike, vitals of the
participant before and after the test, and sweat patch results which include Na+ levels and other such sweat biomarkers, skin impedance (sweat rate), and skin surface temperature. All pieces of information regarding the participant and data collected during the test will be confidentially kept in the office of the research staff on a password-protected computer in which the research staff will be the only ones given the password.

Setting
The exercise stimulation and patch analysis will be conducted at the University of Cincinnati’s College of Education, Human Services, and Criminal Justice (CECH) in the Rashig Human Performance Laboratory with given permission of the lab director.

Estimated Period of Time to Complete the Study
Each test should only take one hour for the subject to complete. The study will last 6 months from initial subject testing to final analysis and write-ups of sweat patch efficacy.

Human Subjects
Subjects are included in this study if they are healthy, between the ages of 18-55 years old and able to give written informed consent. Study participants will be excluded if they are currently pregnant, have preexisting cardiac/heart conditions, have epilepsy, have Cystic Fibrosis, possess eczema or additional skin conditions on patch application site, have diabetes, have known metal allergies, or have skeletomuscular pain/dysfunction. No vulnerable participants will be included in this study. These criteria have been chosen on the basis that this is a study which will require physical exertion to induce sweating. There are no gender biases in this study, however, as close to an equal amount of men and women are desired for this study. The inclusion and exclusion criteria have been chosen for the safety of participants. A valid driver’s license or other form of identification that clearly displays the subject’s date of birth will be used to confirm age requirements before participation in the study. The area of patch applications will be thoroughly examined before application of device. Verbal confirmation will be given by the subject to the best of his or her knowledge to validate the subject does not possess one of the above mentioned conditions which would exclude him/her from the study.

Up to 20 patients will be enrolled in this study at this site. Approximately 20 patients at 1 site in the U.S. will be enrolled in the study.

Subjects will be recruited from the student population at the University of Cincinnati via word of mouth. Upon confirmation by the participant that they would like to participate in this study, the participant will receive and e-mail indicating their testing time and location. They will also be instructed to come in on an empty stomach (no food for at least 1 hour before the test) and will be asked to not have consumed caffeine prior to the test. No participant will be the student of the PI.
Risk/Benefit Assessment
This study is not in any way meant to be more of a risk than a typical exercise session a subject would undergo on his or her own time.

<table>
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<tr>
<th>Risk or Discomfort</th>
<th>Level</th>
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<tr>
<td>Exercise induced fatigue/muscle tension, soreness, or fatigue associated with 20-30 minute active period with resistance selected by subject</td>
<td>Minimal to moderate</td>
</tr>
<tr>
<td>Raised heart rate/cardiac irregularities associated with 20-30 minute active period</td>
<td>Minimal to moderate</td>
</tr>
<tr>
<td>Shortness of breath/respiratory discomfort</td>
<td>Minimal to moderate</td>
</tr>
<tr>
<td>Patch adhesive irritation</td>
<td>Minimal</td>
</tr>
<tr>
<td>Battery short out due to sweat interference</td>
<td>Minimal</td>
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</table>

Exercise Testing
Subjects will be monitored every 5 minutes during the testing procedure. During the exercise portion subjects will be asked every 5 minutes to confirm they are not excessively short of breath, they do not feel skeletomuscular pains, and/or are able to continue with confidence of performance. Validation of subject’s physical confirmation will be recorded at each question time. The stationary bikes used for this study have built-in heart rate monitors that will constantly display the subject’s heart rate throughout the test. The bike is a Monarch Leg Ergometer. It has a Polar Monitor for heart rate interference. Heart rate will be recorded every 5 minutes. Though the subjects will be asked to increase resistance and/or peddling rate in order to simultaneously increase sweat rate, the subject can choose whether or not to do so with no consequence. Increasing sweat flow will allow for optimal detection of patch efficacy, but will not de-validate the results.

Sensor
The research team will also monitor the site of the patch every 5 minutes for irritation and record these results. If any irritation occurs above minimal at any point during the testing procedure, the test will be terminated immediately without question. If any issues with battery pack occur patch will immediately shut off. The battery used in this patch to power the wireless communication is the same type of battery found in most cellular phones. No direct contact with the subject to the battery exists, but excessive sweat may cause interference with current and will cause the patch to automatically cease working for preventative measures.

Payment
Subjects will receive a $10 gift card to a local restaurant. If the subject opts to, at any point, terminate the test he/she will still be issued their gift card.

Subject Costs
There are no costs to the subjects.

Consent Form
Testing of Novel On-Skin Sweat Patch Through Metabolic Sweat Induction_ICD