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A pilot study to compare the efficacy of Pilocarpine Microneedles with iontophoresis method for sweat induction in healthy human subjects

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PROTOCOL TITLE:
A pilot study to compare the efficacy of Pilocarpine Microneedles with iontophoresis method for sweat induction in healthy human subjects

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Center for Cystic Fibrosis and Airway Inflammation Research (CF-AIR) Pilot Grant

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1. Study Summary

Study Title	A pilot study to compare the efficacy of Pilocarpine Microneedles with iontophoresis method for sweat induction in healthy human subjects
Study Design	Phase 1 Clinical Pilot study – cross sectional
Primary Objective	To compare the sweat volume from application of Pilocarpine Microneedle patch to the forearm of healthy adult subjects with that of standard of care method using pilocarpine iontophoresis
Secondary Objective(s)	To measure the sweat chloride concentrations from the sweat samples obtained from the application of Pilocarpine Microneedle patch to the forearm of healthy adult subjects and compare them with measurements from standard of care method using pilocarpine iontophoresis
Research Intervention(s)/Interactions	To perform sweat testing on forearms with standard pilocarpine iontophoresis method and microneedle patch method
Study Population	Healthy adults ≥18 years of age
Sample Size	50 subjects
Study Duration for individual participants	1 day
Study Specific Abbreviations/ Definitions	AE – Adverse Event/Adverse Experience CF – Cystic Fibrosis CFR – Code of Federal Regulations CHOA – Children's Healthcare of Atlanta CTACE – Common Terminology Criteria for Adverse Events FDA – Food and Drug Administration GCP – Good Clinical Practice HIPAA – Health Insurance Portability and Accountability Act IB – Investigator's Brochure ICF – Informed Consent Form IRB – Institutional Review Board N – Number (typically refers to subjects) PHI – Protected Health Information PI – Principal Investigator REDCap – Research Electronic Data Capture SAE – Serious Adverse Event SOP – Standard Operating Procedure
Funding Source (if any)	Center for Cystic Fibrosis and Airway Inflammation Research (CF-AIR)

2. Objectives

The main objective of this study is to demonstrate the safety and feasibility of using pilocarpine microneedles for sweat induction during the process of sweat testing in healthy human adult subjects (≥18 years of age) and comparing it to the standard method of sweat induction (Pilocarpine iontophoresis) in terms of sweat volume collected and sweat chloride concentrations.

3. Background

The sweat test remains the gold standard test for the diagnosis of Cystic Fibrosis (CF). This test is performed in clinical labs and hospitals around the world by clinical personnel for collecting sweat from the forearms to measure the sweat chloride concentration. The current technique for sweat testing has remained unchanged since it was first standardized in the 1960s. Performing this test in newborn infants who have a positive initial newborn screen for CF is clinically very important, as early diagnosis and treatment has helped to improve long-term growth and respiratory outcomes[1]. However, only accredited CF centers generally have the ability to carry out sweat tests. In addition, a common clinical problem encountered in all CF centers is the collection of inadequate volumes of sweat during the testing of young children, which necessitates repeat testing. This can lead to delays in diagnosis and treatment, and also causes significant anxiety[2] for the parents of the newborn who are anxiously waiting to find out if their child has a chronic life-limiting condition[3].

This failure in collecting adequate sweat is especially common in infants less than 3 months of age, and has been shown to be related to several factors such as gestational age[4], ethnicity[5] and skin factors[6]. While newborns are capable of producing sweat, the failure of sweat collection is mostly due to inadequate stimulation of sweat production by iontophoretic delivery of pilocarpine using electricity. Pilocarpine is a cholinergic agonist medication that has been in clinical use since 1874 and it stimulates the sweat glands to produce more sweat. The current sweat testing method (Figure 1) uses a gel disc containing Pilocarpine that drives the medication into the skin with a small electric current (iontophoresis) followed a 30-minute period of sweat collection. Given that it is such an important clinical test, there is an unmet medical need to improve the current testing method and develop more reliable alternatives to induce sweating to ensure adequate sample collection that all CF centers are able to carry out.

In collaboration with Dr. Prausnitz from Georgia Tech, we have developed microneedle (MN) patches to administer pilocarpine to induce sweating in a simple, reliable way without electrical current that does not need special training and does not require sophisticated iontophoretic equipment. As shown in Figure 2, each MN patch contains an array of solid, water-soluble, micron-scale needles that encapsulate the medication[7]. Upon application to skin, the MNs penetrate into the skin's upper layers and dissolve in

the interstitial fluid to release the loaded drugs. MN patches are painless and can be administered with little or no training[8].

MN patches have been extensively studied at Georgia Tech and elsewhere, mostly for vaccination and systemic drug delivery, including successful clinical trials[9]. Pilocarpine delivery has been studied using metal microneedles coated with pilocarpine for administration to the eye for its cholinergic effects[10] and as a drug-free pretreatment of the skin to create micropores to increase efficiency of pilocarpine delivery by conventional iontophoresis[11]. We have developed MN patches that deliver pilocarpine to skin to simplify reliable sweat induction for CF sweat testing without the use of electrical current. Therefore, the impact of our work is to translate a cutting-edge technology (MN patches) to address an unmet medical need (reliable sweat testing) to increase test reliability, enable faster medical treatment, decrease costs and reduce parental anxiety.

Figure 1: Current Standard of Care Sweat Testing Procedure. (A) Pilocarpine gel discs (arrows) placed on positive and negative electrodes. (B) Pilocarpine iontophoresis using 1.5mA current to drive pilocarpine into the skin at the red electrode. (C) Collection of sweat from the stimulated area of the skin on the forearm in a newborn.

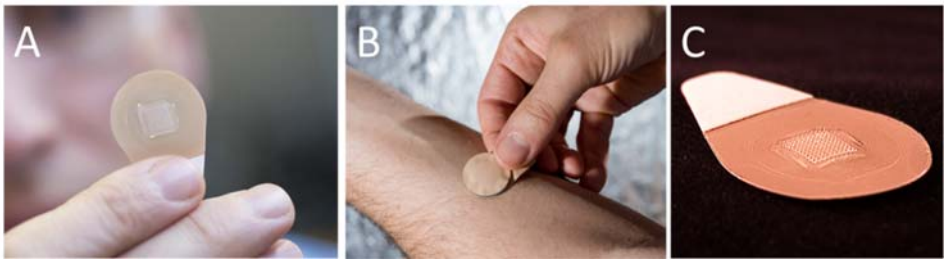


Figure 2. Microneedles patch. (A) A patch with a square array of microneedles in the center. (B) a microneedle patch being applied to the skin. (C) A magnified view showing a patch with an array of 100 microneedles.

Because the pilocarpine microneedle patch is a drug-device combination, it is important to understand the salient characteristics of pilocarpine. The next section provides a

detailed overview of pilocarpine – its pharmacologic properties, doses, indications for use and adverse effects that have been reported in humans.

Pilocarpine

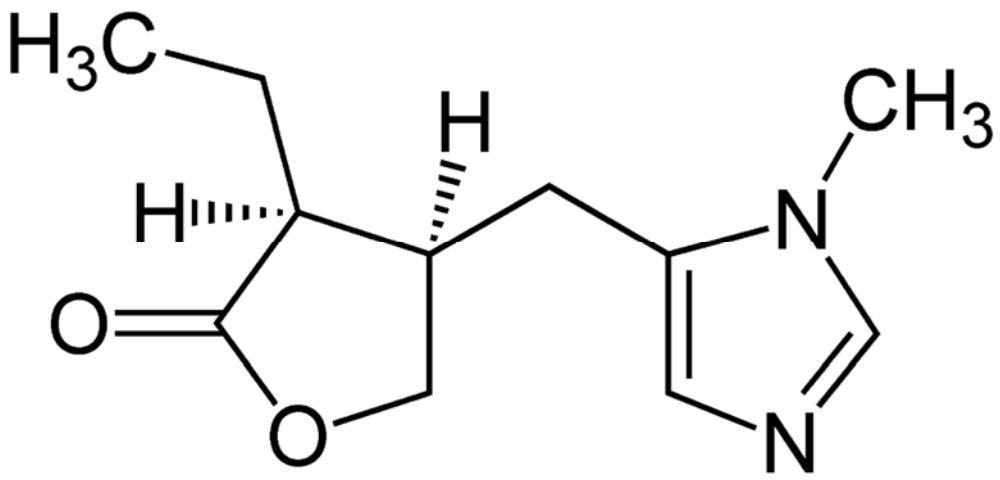


Figure 3: Chemical structure of Pilocarpine

Source of Pilocarpine

Pilocarpine is an alkaloid derived from the plant belonging to genus *Pilocarpus microphyllus* that grows in South America. Although the natives of Brazil had long known that the chewing of leaves of *Pilocarpus* plants caused salivation, the active compound, pilocarpine, was isolated only in 1875 and shown to affect the pupil and sweat and salivary glands.

Pilocarpine is extracted from the powdered leaf material in a multi-step process. First the material is treated with ethanol acidified with hydrochloric acid, and the solvents removed under reduced pressure. The resultant aqueous residue is neutralized with ammonia and put aside until the resin has completely settled. It is then filtered and concentrated by sugar solution to a small volume, made alkaline with ammonia, and finally extracted with chloroform. The solvent is removed under reduced pressure to create pilocarpine powder which can be used to make pills or eye drops.

Physicochemical Properties of Pilocarpine

Pilocarpine is soluble in water, alcohol and chloroform. It is sparingly soluble in ether, benzene. Pilocarpine is hygroscopic in nature and sensitive to light. It is commercially available in powder and liquid suspension form.

Molecular Weight: 271.273 g/mol

Melting Point: 173.5-174 degree Celsius

Log P: 1.1

pKa: 1.6, 1.7

Mechanism of action

Pilocarpine has a dominant muscarinic action but is a partial rather than full agonist; the sweat glands are particularly sensitive to pilocarpine.

Systemically, pilocarpine increases the effect of parasympathetic nervous system and decreases the heart rate. As a muscarinic receptor agonist, pilocarpine produces vascular relaxation and conjunctival vasodilation.

In the eye, Pilocarpine directly stimulates cholinergic receptors causing miosis (by contraction of the iris sphincter), loss of accommodation (by constriction of ciliary muscle), and lowering of intraocular pressure (with decreased resistance to aqueous humor outflow). Therefore, it used for reduction of elevated intraocular pressure (IOP) in open-angle glaucoma or ocular hypertension; management of acute angle-closure glaucoma; induction of miosis (FDA approved in pediatric patients and adults) and prevention of postoperative elevated IOP associated with laser surgery (FDA approved in adults)

When taken in the pill form, it binds to muscarinic (cholinergic) receptors, causing an increase in secretion of exocrine glands (such as salivary and sweat glands) and increase tone of smooth muscle in gastrointestinal and urinary tracts. Its main clinical use in the pill form is for the treatment of xerostomia (dry mouth) caused by radiation therapy in patients with head and neck cancer and from Sjögren's syndrome. This is because Pilocarpine stimulates the salivary gland and stimulates the salivary flow.

When applied to the skin, Pilocarpine stimulates the muscarinic receptor (M1) of myoepithelial cells that surrounds the secretory coils of sweat gland and cause contraction of myoepithelial cells and facilitates secretion of sweat.

Pharmacokinetics

Onset of action: 20 minutes; Maximum effect: 1 hour

Duration: 3 to 5 hours

Half-life elimination: 0.76 to 1.35 hours; mild to moderate hepatic impairment: 2.1 hours

Time to peak, serum: 0.85 to 1.25 hours (increased to 1.47 hours with a high-fat meal)

Excretion: Urine

In the eye,

Onset of action: Miosis: 10 to 30 minutes; Intraocular pressure reduction: 1 hour.

Duration: Miosis: 4 to 8 hours; Intraocular pressure reduction: 4 to 14 hours.

Pharmacodynamics

Pilocarpine is metabolized by the liver and its dose needs to be adjusted in individuals with liver disease. It is eliminated by the kidneys and its excretion can be accelerated by acidification of the urine to trap the cationic form in the urine. The characteristics of oral pilocarpine are fast onset, short duration of action and variable therapeutic dose required by patients. More than half of the oral dose is excreted in urine as unchanged drug.

Clinically used doses of Pilocarpine (Source: Lexicomp)

A. Pill Form

Xerostomia: Oral:

Associated with head and neck cancer: Initial: 5 mg 3 times daily; may titrate dose based on response and tolerability; usual dosage range: 15 to 30 mg/day; maximum: 10 mg/dose

Sjögren syndrome: 5 mg 4 times daily

B. Eye Drops

Dosing: Pediatric

Glaucoma, open-angle; elevated intraocular pressure:

Infants and Children <2 years: Ophthalmic: 1% Solution: Instill 1 drop into the affected eye(s) 3 times daily

Children ≥2 years and Adolescents: **Note:** Strength of solution and frequency of instillation dependent on degree of pressure elevation and patient miotic response; individualize therapy. Ophthalmic: 1%, 2%, or 4% solution: Instill one drop into the affected eye(s) up to 4 times daily; for pilocarpine-naive patients, initiate therapy with the 1% concentration

Glaucoma, acute angle closure:

Infants and Children <2 years: Ophthalmic: 1% solution: Instill 1 drop into the affected eye(s) 3 times daily

Children ≥2 years and Adolescents: Ophthalmic: 1% or 2% solution: Initial: Instill 1 drop into the affected eye(s) up to 3 times over a 30-minute period; pretreatment with secretory suppressant and hyperosmotic agent may be required to lower IOP below 50 mm Hg and relieve iris ischemia. If laser iridoplasty or iridomy required to break the attack, instill 1 drop of 4% solution

prior to the procedure; following laser iridoplasty, instill 1 drop of 1% solution 4 times daily until an iridotomy can be performed.

Miosis induction; prior to goiniotomy or trabeculectomy:

Infants and Children: Ophthalmic: 1% or 2% solution: Instill 1 drop into the eye(s) 15 to 60 minutes prior to surgery

Adolescents: Ophthalmic: 1%, 2%, or 4% solution: Instill 1 or 2 drops (5 minutes apart) into the affected eye(s)

Dosing: Adult

Elevated intraocular pressure: Ophthalmic:

Open-angle glaucoma or ocular hypertension: Instill 1 drop of 1%, 2%, or 4% solution into the affected eye(s) up to 4 times daily; initiate pilocarpine-naive patients on the 1% concentration. **Note:** Strength of solution and frequency of instillation dependent on degree of pressure elevation and patient miotic response.

Angle-closure glaucoma, acute: Instill 1 drop of 2% solution into the affected eye as part of a 4-drug regimen; may repeat in 30 to 60 minutes if intraocular pressure remains elevated (eg, >40 mm Hg). **Note**: Reserve medical management for emergency situations when an assessment by an ophthalmologist will be delayed by ≥1 hour (Pokhrel 2007).

Miosis: Ophthalmic: Instill 1 drop (or 2 drops 5 minutes apart) of 1%, 2%, or 4% solution into the affected eye(s).

Prevention of postoperative elevated intraocular pressure: Ophthalmic: Instill 1 drop (or 2 drops 5 minutes apart) of 1%, 2%, or 4% solution into the affected eye(s) 15 to 60 minutes prior to surgery.

Commercially Available Pilocarpine Brand Names and Products

Eye Drops:

Isopto Carpine: 1% (15 mL); 2% (15 mL); 4% (15 mL)

Generic: 1% (15 mL); 2% (15 mL); 4% (15 mL)

Pills:

Salagen: 5 mg

Salagen: 7.5 mg [contains fd&c blue #2 aluminum lake]

Generic: 5 mg, 7.5 mg

Side effects of Pilocarpine therapy

Eye Drops:

• Tearing
• Burning
• Blurred vision
• Decreased night vision

Systemic:

The following adverse drug reactions and incidences are derived from product labeling unless otherwise specified.

>10%:

Cardiovascular: Flushing (8% to 13%)

Central nervous system: Chills (3% to 15%), dizziness (5% to 12%), headache (11%)

Gastrointestinal: Nausea (6% to 15%)

Genitourinary: Urinary frequency (9% to 12%)

Neuromuscular & skeletal: Weakness (2% to 12%)

Respiratory: Rhinitis (5% to 14%)

Miscellaneous: Diaphoresis (29% to 68%)

1% to 10%:

Cardiovascular: Edema (<1% to 5%), facial edema, hypertension (3%), palpitation, tachycardia

Central nervous system: Pain (4%), fever, somnolence

Dermatologic: Pruritus, rash

Gastrointestinal: Diarrhea (4% to 7%), dyspepsia (7%), vomiting (3% to 4%), constipation, flatulence, glossitis, salivation increased, stomatitis, taste perversion

Genitourinary: Vaginitis, urinary incontinence

Neuromuscular & skeletal: Myalgias, tremor

Ocular: Lacrimation (6%), amblyopia (4%), abnormal vision, blurred vision, conjunctivitis

Otic: Tinnitus

Respiratory: Cough increased, dysphagia, epistaxis, sinusitis

Miscellaneous: Allergic reaction, voice alteration

<1%: Abnormal dreams, abnormal thinking, alopecia, angina pectoris, anorexia, anxiety, aphasia, appetite increased, arrhythmia, arthralgia, arthritis, bilirubinemia, body odor, bone disorder, bradycardia, breast pain, bronchitis, cataract, cholelithiasis, colitis, confusion, contact dermatitis, cyst, deafness, depression, dry eyes, dry mouth, dry skin, dyspnea, dysuria, ear pain, ECG abnormality, eczema, emotional lability, eructation, erythema nodosum, esophagitis, exfoliative dermatitis, eye hemorrhage, eye pain, gastritis, gastroenteritis, gastrointestinal disorder, gingivitis, glaucoma, hematuria,

hepatitis, herpes simplex, hiccup, hyperkinesias, hypoesthesia, hypoglycemia, hypotension, hypothermia, insomnia, intracranial hemorrhage, laryngismus, laryngitis, leg cramps, leukopenia, liver function test abnormal, lymphadenopathy, mastitis, melena, menorrhagia, metrorrhagia, migraine, moniliasis, myasthenia, MI, neck pain, photosensitivity reaction, nervousness, ovarian disorder, pancreatitis, paresthesia, parotid gland enlargement, peripheral edema, platelet abnormality, pneumonia, pyuria, salivary gland enlargement, salpingitis, seborrhea, skin ulcer, speech disorder, sputum increased, stridor, syncope, taste loss, tendon disorder, tenosynovitis, thrombocythemia, thrombocytopenia, thrombosis, tongue disorder, twitching, urethral pain, urinary impairment, urinary urgency, vaginal hemorrhage, vaginal moniliasis, vesiculobullous rash, WBC abnormality, yawning

Drug Interactions

Acetylcholinesterase Inhibitors: May enhance the adverse/toxic effect of Cholinergic Agonists. Specifically, cholinergic effects may be enhanced or increased. *Risk C: Monitor therapy*

Beta-Blockers: May enhance the adverse/toxic effect of Cholinergic Agonists. Of particular concern are the potential for cardiac conduction abnormalities and bronchoconstriction. *Risk C: Monitor therapy*

Cimetropium: Cholinergic Agonists may diminish the anticholinergic effect of Cimetropium. *Risk C: Monitor therapy*

Sincalide: Drugs that Affect Gallbladder Function may diminish the therapeutic effect of Sincalide. Management: Consider discontinuing drugs that may affect gallbladder motility prior to the use of sincalide to stimulate gallbladder contraction. *Risk D: Consider therapy modification*

Food Interactions

Fat decreases the rate of absorption, maximum concentration and increases the time it takes to reach maximum concentration. Management: Avoid administering with a high-fat meal.

Pregnancy Considerations

Adverse events have been observed in some animal reproduction studies.

Breast Feeding Considerations

It is not known if pilocarpine is excreted in breast milk. Due to the potential for serious adverse reactions in the nursing infant, the manufacturer recommends a decision be made to discontinue nursing or to discontinue the drug, taking into account the importance of treatment to the mother.

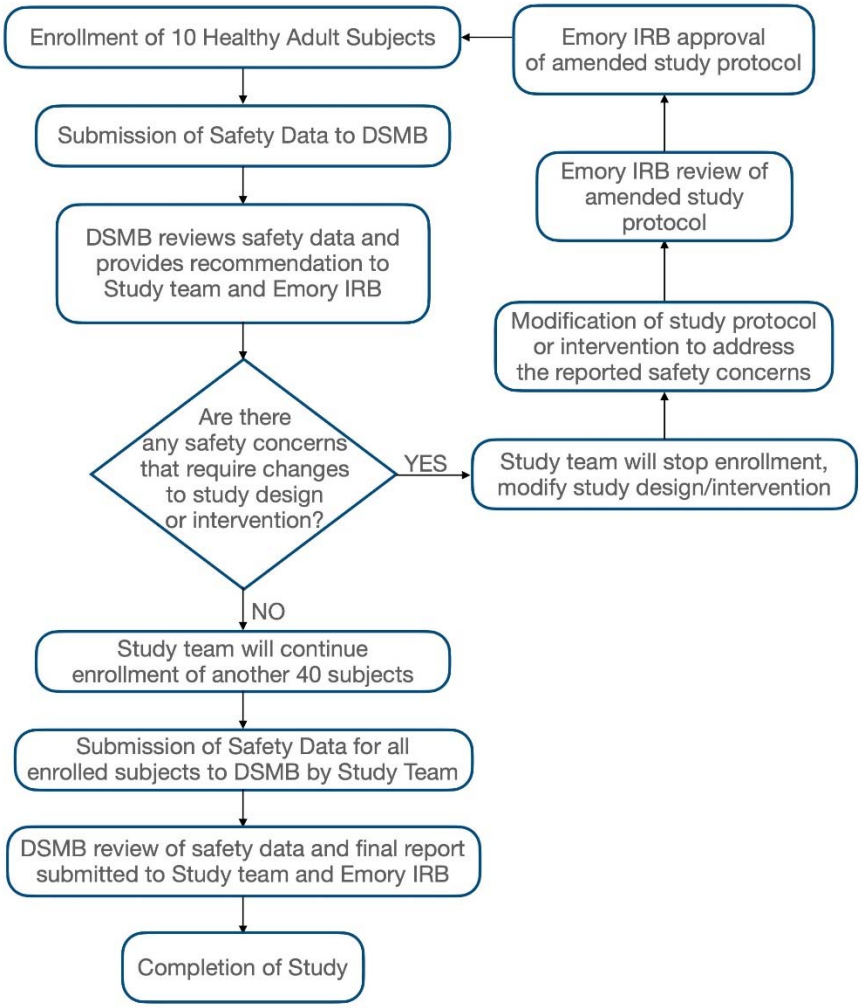
FDA Approval Date

Pilocarpine Hydrochloride: March 22, 1994

4. Study Design

This is a cross sectional pilot study which will be conducted in a phased manner (Figure 4). The first phase of the study will include the enrollment of 10 healthy adult subjects who will undergo testing to assess the safety and efficacy of pilocarpine microneedle patch. The safety data from the first 10 subjects will be submitted to the Data and Safety Monitoring Board (DSMB) that will review the reported adverse events during testing. The DSMB will independently assess this safety data and provide their recommendations on whether it is safe to continue the study or not. These recommendations will be submitted to the Emory IRB. If there are no safety concerns reported by DSMB, then the second phase of the study will begin where the study team will continue enrollment of an additional 40 subjects to complete testing. After the enrollment and testing of 50 subjects, the study will be completed and another report of safety data will be submitted to the DSMB at the end of the study. The DSMB will review this final report and their final recommendations will be shared with Emory IRB and study team.

Figure 4: Study Design Flowchart



If there are any safety concerns reported by the DSMB, then these will be addressed by the study team with an appropriate modification of the study design or intervention. This will be followed by submission of an amended study protocol to the IRB to reflect the changes made to the study protocol. After approval of the amended study protocol, the study team will conduct the revised testing procedure on 10 subjects and submit safety data to the DSMB again for their review and approval.

5. Study Endpoints

The study endpoint will be when the primary and secondary outcome measures are obtained for all the enrolled participants and data analysis has been completed.

The main outcome measures for the study are:

5.1 *To compare the sweat volume from application of Pilocarpine Microneedle patch to the forearm of healthy adult subjects with that of standard of care method using pilocarpine iontophoresis*

5.2 *To measure the sweat chloride concentrations from the sweat samples obtained from the application of Pilocarpine Microneedle patch to the forearm of healthy adult subjects and compare them with measurements from standard of care method using pilocarpine iontophoresis*

6. Study Intervention/Investigational Agent

The study involves the use of pilocarpine for its approved indication for sweat induction during the clinical procedure for sweat testing in human subjects. The pilocarpine will be administered in the form of an agar gel disc using iontophoresis (Standard Procedure) on one arm of the subject, while the other arm will receive pilocarpine microneedles (Experimental Procedure) without any iontophoresis. This will allow a direct comparison of the two methods in terms of the efficiency of induction of sweat production in human subjects and whether the sweat chloride concentrations in the sweat samples obtained by the two methods are comparable or not. In addition, a control microneedle patch without any Pilocarpine will be placed on the left forearm to assess the effect of the microneedle patch use. The subsequent steps for the collection of sweat after completing the induction will be done with the same method for both arms using a macroduct collector at each site (Standard Procedure). There will be no sweat collection performed at the site the control microneedle patch. Pictures of the skin sites will be taken before and after the interventions at each site.

The study team will plan to enroll study subjects of all ethnicities or racial backgrounds, so that the impact of this intervention can be studied in individuals with different skin characteristics.

Because this study involves an approved drug (Pilocarpine) that has been in clinical use for application to the skin for the purpose of sweat testing, we think that this project will not require an Investigational New Drug (IND) application. This has been discussed at length with several regulatory experts and FDA and the conclusion of these discussions was that the drug component is exempt from an IND under 21 CFR 312.2(b), and the device component is exempt from an IDE under 21 CFR 812.2(b), since all the related conditions in those two provisions are met by our device and study design.

We have reviewed the guidelines from the Food and Drug Administration (FDA)[12] for determining whether certain human research studies can be conducted without an IND (From FDA website: https://www.federalregister.gov/documents/2015/10/30/2015-27729/investigational-new-drug-applications-determining-whether-human-research-studies-can-be-conducted).

This document provides the criteria that FDA uses to determine if any human subjects research study involving a drug needs an IND or not. We have extracted the 6 main criteria from this FDA advisory document and provided an explanation of how our study meets those criteria set forth by the FDA.

1) The drug product is lawfully marketed in the United States.

For our proposed study, we are planning to use Pilocarpine, which is available for use in United States and is approved by the FDA for standardized induction of sweat production during sweat testing. The Sweat Test is the gold standard clinical test for the diagnosis of Cystic Fibrosis, the most common lethal genetic disease in Caucasians. This test involves collection of a small amount of sweat from the forearms after the application of an agar gel disc containing pilocarpine with iontophoresis using a small electric current (1.5mA) for 5 minutes.

Pilocarpine is a cholinergic agonist agent with the ability to induce sweat production from eccrine human sweat glands based on its ability to stimulate muscarinic receptors in the sweat gland ducts. After the stimulation phase with pilocarpine, the sweat produced by the skin on the forearm is collected and analyzed for its chloride concentration. Improving upon the current technique of sweat testing will reduce inaccurate or inadequate results and improved diagnostic certainty in newborn babies that are screened for Cystic Fibrosis at birth across United States and most of Europe as well.

2) The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication and there is no intent to use it to support any other significant change in the labeling of the drug.

This study will not be reported to the FDA as a case-control study as we are planning to study the feasibility of pilocarpine delivery to the skin using microneedle patches in

healthy subjects only. The results of this study will compare the effectiveness of the microneedle method of sweat induction with the standard of care pilocarpine iontophoresis method in human subjects and will not change the labeling of the drug. The sweat collection phase and analysis of chloride concentration will remain in the same and we will simply compare the volume of sweat collected between the standard method of sweat induction (using iontophoresis) with our proposed microneedle-based approach.

3) In the case of a prescription drug, the investigation is not intended to support a significant change in the advertising for the drug.

This study and the data being collected from it will not be used in any advertising materials for the drug. The same drug (pilocarpine) is being used for sweat stimulation in this study and we are simply comparing the effectiveness of another mode of delivery of this medication. If found effective, it will not change the use of this drug for the purpose of sweat testing.

4) The investigation does not involve a route of administration, dose, patient population, or other factor that significantly increases the risk (or decreases the acceptability of the risk) associated with the use of the drug product (21 CFR 312.2(b)(1)(iii)).

The delivery of pilocarpine for sweat induction using the microneedle approach does not change the route of administration (transcutaneous) for the purpose of sweat testing. The dose used in microneedles is significantly lower and does not increase the risk for the subjects being tested. The patient population that this test is applicable to remains the same – all subjects that are being evaluated for Cystic Fibrosis. There is no change in the risk profile, as the drug is being applied to the skin for the stimulation of the sweat glands on the forearm. Since our proposed microneedle method does not involve the use of electric current, there will actually be a lower risk of tingling sensations and rarely skin burns that sometimes occur with the iontophoresis procedure. The standard pilocarpine iontophoresis method of sweat induction has been rarely reported to cause small burns on the skin surface related to the use of electric current. Our proposed method eliminates this risk of burns completely.

In particular, the FDA advisory document (page 5)[12] provides the following explanation regarding the risk of the modifications made in the proposed study:

In responding to comments asking FDA to clarify to what extent a sponsor could change the marketed drug product or conditions of use and still be exempt from the IND regulations, FDA stated that:
The exemption was not intended to require an investigator to use the drug in exactly the same dosage form, dosage levels, and patient populations described in the marketed labeling for the product, but rather to permit changes to the

lawfully marketed drug product that do not increase the risks . . . over the risk presented by use of the product in conformance with its marketed labeling

Since our proposed study using microneedle-based delivery of pilocarpine reduces the risk associated with standard of care iontophoresis method, it satisfies this criterion as well.

5) The investigation is conducted in compliance with the requirements for review by an IRB (21 CFR part 56) and with the requirements for informed consent (21 CFR part 50).

The study will be submitted for evaluation by Emory Institutional Review Board and all the stated rules and regulations will be followed by the principal investigator during the conduct of the study. An informed consent will be signed by every participant prior to undergoing any testing and the subjects will be informed about the voluntary nature of the study and that they have the right to withdraw consent for this study at any given time. Their data will be anonymized and stored in a HIPAA compliant manner in a password protected secure network computer.

6) The investigation is conducted in compliance with the requirements of § 312.7 (i.e., the investigation is not intended to promote or commercialize the drug product).

The study will be conducted by researchers from Emory University and Georgia Institute of Technology without any intention of promoting any commercial drug products.

Drug/Device Handling: The supplies for the Standard Procedure will be obtained from the manufacturer directly and stored according to their specifications. The pilocarpine microneedles that will be used for the experimental procedure will be manufactured at Global Center for Medical Innovation (GCMI). This is not part of Georgia Tech. It is an independent institution. The patches will be delivered to Emory University by a member of Dr. Prausnitz lab. The patches will then be stored at room temperature at Emory University in a secure location until it is used for a study subject.

7. Procedures Involved

Informed consent will be obtained by an IRB-approved member of the study staff. The process of informed consent will begin with a verbal description of the experimental tasks. Participants will then have an opportunity to read the informed consent form that explains in more detail the requirements for participation, including protections of privacy and confidentiality. Participants will be told of their rights to discontinue participation at any time and that there will be no immediate benefit to participating and no disadvantage to discontinuing. Participants will be given contact numbers for the PI and the Executive Director of the Human Research Protection Office should they have

any questions or concerns regarding their participation. Informed consent documentation will take place in a private setting convenient to the participant.

Once the consent is obtained, the patient will undergo sweat testing. The testing will be performed with the subject resting comfortably on a chair and total duration of testing will be 45 minutes. The right forearm will be used for pilocarpine iontophoresis and the left forearm will be used for microneedle-based stimulation for a period of 5 minutes. A control microneedle patch without pilocarpine will also be placed on the left forearm to assess for any microneedle patch related changes. After the stimulation phase, collection of sweat will be performed on each forearm simultaneously for a period of 30 minutes (excluding the control microneedle patch site). At the end of the 30-minute sweat collection period, the collection device will be removed and the study visit will be completed. All the collected sweat samples (2 for each subject) will be frozen for later analysis of the sweat volume and chloride concentration.

The pilocarpine microneedles that will be used for the experimental procedure will be manufactured at Global Center for Medical Innovation (GCMI). This is not part of Georgia Tech. It is an independent institution. The pilocarpine-loaded MN patches are fabricated by a two-step molding process using polydimethylsiloxane (PDMS) molds with some modification on an established method. The first casting solution is a mixture of 10% (w/v) pilocarpine nitrate, 10% (w/v) poly (vinyl alcohol) and 5% (w/v) sucrose, which is prepared by mixing the different components in water at the desired ratio. This solution is then cast on PDMS molds under vacuum to facilitate filling the solution into the molds. After 20 min, excess solution is removed, and the filled molds are centrifuged at 5000 rpm for 20 min to dry the drug loaded MNs. The second casting solution containing 20% (w/w) polystyrene in 1, 4-dioxane is then cast on the filled PDMS molds under vacuum. The mold is kept under vacuum for another 3 h to dry the solution at room temperature, and then further dried at 40°C overnight before demolding the MN patches by adhesive tape. Each patch is immediately stored with desiccant in individual sealed pouches prior until use.

8. Data and Specimen Banking

Data for the study participants will be stored in the online REDCap (Research Electronic Data Capture) Database with only de-identified information being used for statistical analyses. REDCap is a secure, web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources. Access to this data will be limited to the study investigators only. Sweat samples may be stored frozen at Emory Children's Center for future tests. All the used microneedle patches will be sent to Prausnitz lab to measure the amount of Pilocarpine delivered into the skin and then the patches will be destroyed as per the steps outlined in Appendix 8.

9. Sharing of Results with Participants

The study team does not plan to share the results of the sweat sample collection. Sweat samples will be tested for chloride concentration and volume. The summary results will be posted on clinicaltrials.gov website.

10. Study Timelines

Participants will only need to take part in one study visit totaling 45 minutes. The study team anticipates enrolling all study participants over the course of 6 months. Completion of all study related procedures, including data analysis, is expected to take approximately 12 months. No follow up visits will be needed for any of the study participants.

11. Inclusion and Exclusion Criteria

Inclusion Criteria:

- Age ≥18 years
- Signed a written informed consent
- Not taking any medications
- No known medical diagnoses or chronic conditions

Exclusion Criteria:

- Age <18 years
- Family history of Cystic Fibrosis
- History of skin disorders (eczema, psoriasis etc.) that could prevent sweat testing on forearms
- Current medication use
- Pregnant women
- Breastfeeding women

12. Local Number of Participants

We will enroll 50 healthy adults aged 18 years or more from the general community with the help of flyers that will be placed at various places at the Emory campus. There will be no exclusion of subjects based on gender or ethnicity.

13. Recruitment Methods

Participants will be recruited through flyers and consent will be obtained during their scheduled study visit. Recruited subjects will not require any follow up visits or additional testing.

14. Withdrawal of Participants

Subjects will only be withdrawn from the study if they are unable to complete the sweat sample collection. After a subject has been withdrawn, the study team will discard incomplete sweat sample collection and no more data will be collected.

15. Risks to Participants

There are some risks associated with both forms of sweat testing.

All study subjects will be warned about the potential risk of skin burns that can occur with the use of the pilocarpine iontophoresis step during the standard-of-care method of sweat testing.

The product insert from the manufacturer detailing this side effect has been added as Appendix 10. This risk is estimated to occur at a rate of about 1 in 50,000 tests. The burns have been reported to be ranging from “tiny black pinholes in the skin” to “crater-like, third degree burns two to three millimeters in diameter”. As per the manufacturer’s product insert, individuals who had these burns had no signs of pain or discomfort during iontophoresis, and the burns are not noticed until after the electrodes are removed. The burns are reported to heal completely in 1-2 weeks with little or no scarring. The manufacturer of pilocarpine iontophoresis also warns about the risk of redness of skin at the electrode site, and in some cases, blister-like welts may form which can persist up to 2-3 hours. However, this standard-of-care method has been approved for use in humans by the FDA.

With the use of pilocarpine microneedle patch, there is a risk of skin irritation or redness that can last for hours after the completion of testing. There is no risk of burns with the pilocarpine microneedle patch. There is no risk of systemic side effects from the exposure to Pilocarpine as the total dose of pilocarpine in each microneedle patch is less than 10% (one-tenth) of the standard adult dose that is used in clinical practice (see section on Pilocarpine doses above on page 8). Not all the pilocarpine that is present on a microneedle patch will be delivered into the skin, and only a fraction of that small dose penetrating the skin may be absorbed into systemic circulation.

Due to the pilot nature of this study, the study will have a Data and Safety Monitoring Board (DSMB) comprised of 3 members that are not affiliated with this study who will review the safety data for the first 10 subjects enrolled. The DSMB charter has been reviewed and signed off on by all the members of the DSMB (Appendix 11).

After the DSMB has reviewed the safety of the pilocarpine microneedle approach in the first 10 subjects, it will provide its recommendations to the study team and this communication will be submitted to Emory IRB as well. If the DSMB recommends

continuation of the study, then further enrollment will be continued to enroll an additional 40 subjects with close monitoring for any unforeseen risks or side effects of the interventions related to this study in the remaining participants. Any unforeseen side effects of the pilocarpine microneedle patch will be reported to the DSMB for review. The DSMB will again review the safety data at the end of the enrollment of the additional 40 subjects to ensure that no additional concerns have arisen during the remainder of the study. The DSMB will provide their recommendations and summary regarding the safety of the pilocarpine microneedle patch being test in this study and this will also be shared with Emory IRB. If there are any safety concerns reported by the DSMB, then these will be addressed by the study team with an appropriate modification of the study design or intervention. This will be followed by submission of an amended study protocol to the IRB to reflect the changes made to the study protocol. After approval of the amended study protocol, the study team will conduct the revised testing procedure on 10 subjects and submit safety data to the DSMB again for their review and approval.

16. Potential Benefits to Participants

16.1 There is no direct benefit to the subjects from participation in this study.

16.2 The study will benefit the advancement of medical knowledge about the usefulness of pilocarpine microneedle patches for the induction of sweating during the procedure of sweat testing.

17. Compensation to Participants

Subjects will not be compensated for participating in this study.

18. Data Management and Confidentiality

Descriptive analysis will be conducted for the study participants to describe their clinical presentation and long-term outcomes. Several steps will be taken to secure the data by ensuring adequate training of the study team members, authorization of access to the study team for the clinical data, password protection, encryption, and separation of identifiers and data during the storage, use, and transmission. Data will be stored on each users' secure servers and not downloaded to external devices, including laptops. The information, even if de-identified, will be destroyed at the expiration of the project.

19. Economic Burden to Participants

There will be no costs to participants that enroll this study.

20. Consent Process

Consent of participants will take place during their scheduled study visit. The research coordinator will first introduce the study to the participant and give the participant ample time to consider enrolling in the study and to ask questions. The subject will receive a copy of their signed consent form.

21. Setting

Study participants will be recruited with the help of study flyers. After participants have been screened based on inclusion and exclusion criteria and informed consent obtained, their study visit will take place in a designated research room located on the first floor of Emory Children's Center. Study samples that are collected will then be taken to the research lab for sample processing.

22. Pilocarpine Microneedle Patch Composition

The pilocarpine-loaded MN patches are fabricated by a two-step molding process using polydimethylsiloxane (PDMS) molds with some modification on an established method. The first casting solution is a mixture of 10% (w/v) pilocarpine nitrate, 10% (w/v) poly (vinyl alcohol) and 5% (w/v) sucrose, which is prepared by mixing the different components in water at the desired ratio. While this is our current formulation, we will investigate other formulations that can increase the pilocarpine content (on a solids basis) and thereby increase the dose of pilocarpine per MN. This solution is then cast on PDMS molds under vacuum to facilitate filling the solution into the molds.

After 20 min, excess solution is removed, and the filled molds are centrifuged at 5000 rpm for 20 min to dry the drug loaded MNs. The second casting solution containing 20% (w/w) polystyrene in 1, 4-dioxane is then cast on the filled PDMS molds under vacuum. The mold is kept under vacuum for another 3 h to dry the solution at room temperature, and then further dried at 40°C overnight before demolding the MN patches by adhesive tape. Each patch is immediately stored with desiccant in individual sealed pouches prior until use.

23. Pilocarpine Microneedle Patch Manufacturing and Characterization

The steps involved in Microneedle patch manufacturing have been outlined in Appendix 01.
The device master record showing all the components that went into the research article is included in Appendix 2. Design Trace matrix is an engineering description of each feature of the microneedle patch and the respective rational for that feature is provided in Appendix 3.

Verification Testing for the research articles was done through several methods. A Mechanical Test Report is included in Appendix 4. Pig skin test report is included in Appendix 5. Process validation report is included in Appendix 6. A visual and dimensional analysis of the microneedle patch is included in Appendix 7.

The process of extraction of Pilocarpine Nitrate through HPLC method is described in Appendix 8.

A detailed drawing of the research article with all the measurements is included in Appendix 9.

24. Pilocarpine Microneedle Patch Measurements and Disposal after use

The process of determining pilocarpine dose delivered per Microneedle patch will be completed at Prausnitz lab. All used patches will be stored in their respective containers and transported back to Prausnitz lab. The patches are then dissolved and the remaining amount of Pilocarpine in the patch will be measured as per the steps outlined in Appendix 8.

25. Appendices

Appendix 1: Manufacturing Procedure
Appendix 2: Device Master Record
Appendix 3: Design Trace Matrix
Appendix 4: Mechanical Test Report
Appendix 5: Pig Skin Report
Appendix 6: Process validation report
Appendix 7: Visual and Dimensional Measurement Report
Appendix 8: HPLC Pilocarpine Nitrate extraction Appendix
9: Detailed Drawing of Research Article
Appendix 10: Risk of Skin Burns from Pilocarpine Iontophoresis – Manufacturer Product Insert
Appendix 11: Data Safety Monitoring Board Charter

26. References

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