PROTOCOL TITLE:

Using a Cold Atmospheric Plasma Device to Treat Molluscum Contagiosum and Verruca Vulgaris in Pediatric Patients

PRINCIPAL INVESTIGATOR:

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NCT05070754

1.0 Objectives / Specific Aims

- Objective: Study the safety and efficacy of uniform nonequilibrium, nonthermal atmospheric pressure plasma (NTAP)/cold atmospheric plasma device (CAP) in the treatment of molluscum contagiosum and vercurra (warts) in children 4-21 years.
- Aim:
  - Measure the changes in size, pain and appearance of verrucae and molluscum after 4, 8 and 12 weeks.
  - Evaluate the safety profile and patient tolerability of the treatment by analyzing potential side effects and pain levels, or lack thereof, with each treatment.
- Hypothesis: NTAP will be an equally efficient and painless alternative to cryotherapy for the treatment of molluscum and warts.

2.0 Background

Molluscum contagiousum (MC) and warts are common viral disease in children. They result from an infection with a double stranded DNA virus (1). Diagnosis is clinical, presenting as smooth, round papules with central umbilication in MC and irregularly surfaced domed lesions in warts. Although benign and generally self-limited, these conditions are contagious and can lead to complications such as inflammation, pruritus, dermatitis, scars and superinfections (2). There is little consensus concerning the management of these conditions. Treatment options include mechanical destruction with cryosurgery or heat, topical irritants, topical vesicants and topical acids (3). These strategies are often irritating and/or painful making application to children very difficult, particularly in cases where many have to be treated at once.

Physical plasma has been considered the fourth state of matter. Biomedical application at lower-temperature plasma have been regarded as one of the most significant opportunities in plasma science. Nonthermal, cold and non-equilibrium plasma synonymously mean plasma that is not in thermodynamic equilibrium because the electron temperature is significantly warmer than heavy species. When the electrons are thermalized their velocity distribution is very different from the ion velocity distribution leading to modification in DNA, proteins and cell membranes. Most NTAP plasmas are generated in helium or argon mixed with a number of reactive gases (7). This creates a low ion energy environment with a rapid reaction response in which UV light and reactive oxygen and nitrogen species induce a focused, transient antiviral or antibacterial milieu (18). NTAP offers direct delivery to the sample surface without the complications of a vacuum chamber to physically burn the tissue and trigger a complex sequence of biological responses in tissue and cells (4). Plasma does not cause lasting damage to the skin or skin functions, indicating a very favorable safety profile for its application in treating cutaneous pathologies (20).

Plasma affects cells in a dosage-dependent manner by disrupting cell-cell adhesion and cell detachment from substrates (12). The short-term exposure temporary causes cell membrane permeabilization, inhibiting cell migration. Longer exposure times and higher intensity plasma induces apoptosis or necrosis by reactive oxygen and nitrogen species. Several pilot studies have confirmed that NTAP induces multiple modes of cell death in human melanoma and hepatocellular carcinoma. The plasma damaged cells begin to repair the damage with releasing growth factors (ex: fibroblast growth factor 2) (12).

In the biomedical field, NTAP has a broad range of applications including disinfection of living tissues, blood coagulation, induction of apoptosis in malignant tissues, a localized modulation of cell adhesion and proliferation and tissue modification of interest in electrosurgery (5-7,9,10). The first small scale clinical studies have shown plasma treatments were well tolerated, painless and, most importantly, without side effects (20-26). In a risk assessment of UV radiation and temperature, it was shown that UV radiation of the plasma is an order of magnitude lower than the minimal erythema dose necessary to produce sunburn on the skin *in vivo*. Additionally, thermal damage of the tissue by the plasma can be excluded since the electrons and ions are not in thermal equilibrium, thereby resulting in "cold," rather than "hot" plasma, which is actually room temperature upon tactile examination. 27).

Multiple modes of NTAP, also known as cold atmospheric plasma (CAP), have been employed in dermatologic clinical studies such as wound healing, onychomycosis, actinic keratosis and viral warts (28). Friedman's case series on warts proved it safety and efficacy in children, reporting no adverse events (blistering, scarring, significant pigmentary alteration or persistent nail changes) or pain reported in treatment (29). A proof-of-concept study on warts in children demonstrated efficacy and exceptional tolerability when treated with plasma as a result of its intracellular calcium influx and viral replication inhibiting effects. In actinic keratosis they had a 70% improvement rate with no discomfort during or after treatment. Onychomycosis had a 50% clinical and 15% mycological cure rate in 13 patients without any side effects. And wound healing of chronic ulcers had decreased bacterial as expected from well-documented inhibitory effects on bacteria and also superior wound healing rates compared to wound care alone. Moreover, it has been shown to be safe and successful in proof-of concept studies exploring its application in the treatment of Verruca vulgaris. Its efficacy in molluscum has not yet been tested.

Given NTAP's outstanding safety profile and painless application (5), its prior proof of concept success in treating common verrucae, and the similarity between verrucae and molluscum, we propose to use NTAP to treat these conditions and expand upon the existing research literature.

If the NTAP device is shown to be a successful, efficacious and painless treatment option for children and adolescents with verrucae and molluscum, this would be a revolutionary and environmentally friendly approach to two of the most common viral infections and chief complaints found during dermatology visits in this age group. The non-toxic, chemical free, waste free, multi-year device results in a significant reduction of effluents, which is beneficial from an economic but also an environmental viewpoint (30).

3.0 Intervention to be studied (if applicable)

We will utilize a floating electrode-dielectric barrier device (FE-DBD) Cold atmospheric plasma (CAP) device on molluscum and warts in children and adolescents 4-21 years (Figure 1). Children and adolescents are targeted because they are the population most affected by painful standard of care therapy. The study will continue until at least 68 warts and 68 molluscum are treated. Each patient will not have more lesions treated than SOC recommendations. Half the lesions will be treated with plasma and the other half with standard of care being cryotherapy for warts and cantharidin for molluscum. A single patient must have minimum of 1 clinically diagnosed lesion treated and maximum of 20 treated. Lesions with a clinical impression of warts or molluscum will be selected. Selection of treatment type (control vs NTAP) will be randomly selected. Lesions

The treatment device in this study generates cold atmospheric plasma. Cold atmospheric plasma has certain properties of plasma, such as ionized gas molecules. To create plasma, a pulse generator supplying 20-kV pulse of 20-ns pulse width at 200 Hz (FPG10-01NM10, FID GmbH, Burbach, Germany) to a 5-mm diameter quartz-covered copper electrode of 10-cm length and 1-mm quartz thickness (5). These nanosecond pulse parameters were chosen to provide sufficient treatment dose at the high level of plasma uniformity required to avoid any tissue damage. We treated the lesions for approximately 1 to 2 minutes each, moving the electrode gently over the treatment area.

After the study treatments are completed, we will analyze the data to see if the treatment was safe and effective.

Non-significant risk device statement (6-19) (Figure 1)

- We are proposing a clinical trial floating electrode-dielectric barrier device (FE-DBD) a Cold Atmospheric Plasma (CAP) device.
- While novel to the medical field and especially to dermatology, there are already a number of publications regarding its use on human skin in adults (20-26) and children (29).
- CAP devices utilize noble gases (such as helium) to deliver plasma state matter to the skin. As its name implies, the generated plasma stream is of near skin temperature and it exists on normal atmospheric pressure. During the generation of the plasma there is no electric contact with the patient. The treatment does not increase skin surface temperature and the used helium gas - the same as used for balloons - being a noble gas does not develop a chemical reaction with the skin. The flow of the gas is slow, thus there is no mechanical effect on the skin.
- Previous clinical studies used these devices on skin surfaces that were denude, eroded or ulcerated and even without intact skin barrier function there were no reported adverse effects. In fact, one study specifically examined the tolerability of plasma treatments of various equipment and found that the plasma was well tolerated and had no adverse effects on the skin surface.
- Based on the principles of the treatment as well as the below referenced prior studies we believe that this non-invasive method of treatment will be performed using equipment that certainly qualifies for a minimal-risk device that will not harm normal skin.

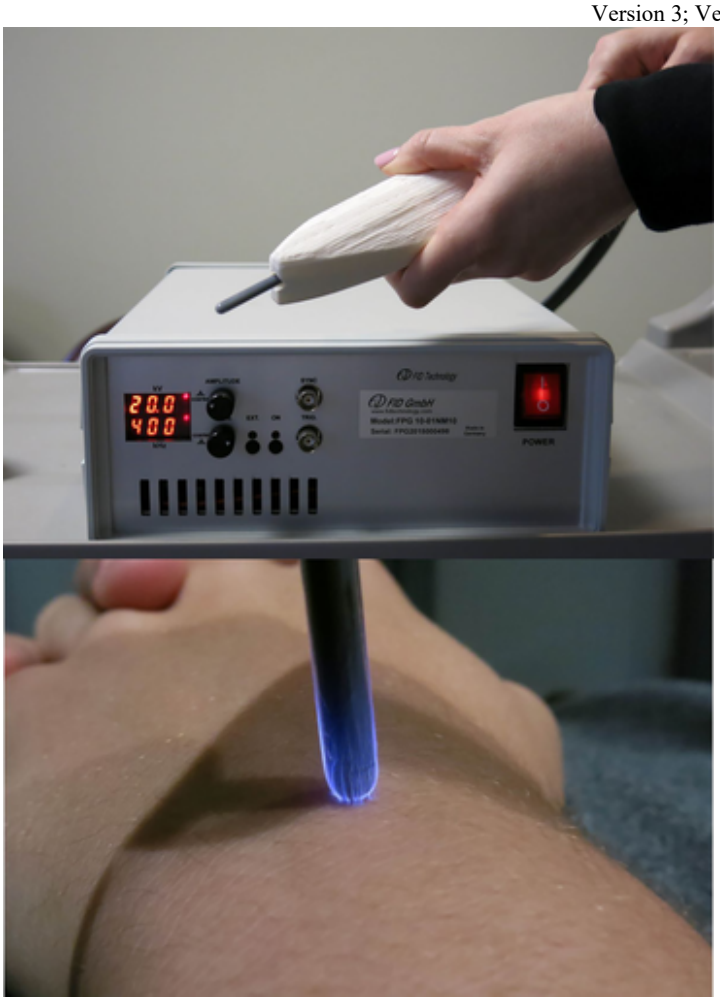

Fig. 1. Floating-electrode DBD plasma. Top panel: pulse generator and hand-held electrode. Bottom panel: Plasma is being generated using the electrode tip. The electrode connected to the pulse generator must be kept near the treated surface. Either the tip or the side of the electrode can be used, but plasma will only form where the gap between the electrode and the target surface does not exceed 1 mm. This device pictured will be used in our trial.

Clinical safety of plasma (28):
Standards are issued by the national regulatory authorities like UV radiation, ozone, nitrogen oxide and electrical current. Assuring compliance to maintained through measuring values for every CAP device and exposure limits. The presence of reactive species raises a concern for the mutagenicity of CAP; however, all devices were found safe within the operating parameters studied. To ensure safety of each CAP device, everyone must be tested on standards developed by the International Organization on Plasma Medical Device and Standardization (IOPMS) with Dr. Alexander Fridman representing the US. IOPMS is considering device safety under the major aspects:

(i) biomedical safety, related to optimization of protocols, suppression of side effects, detailed analysis of clinical studies.
(ii) physical, technological, and electric safety, related to limitations of generation of ozone, UV radiation, leak currents, electromagnetic interaction with other devices in medical environment
(iii) safety aspects related to device (and treatment protocol), sensitivity to device positioning, dosimetry, environmental conditions

Dermalite dermatoscopes will be used to assess diagnosis and resolution. They are approved by the FDA for the study indication. Dermoscopy, also known as dermatoscopy, epiluminescence microscopy, incident light microscopy, or skin surface microscopy, is performed using a handheld instrument called a dermatoscope or dermoscope, which has a transilluminating light source and standard magnifying optics (10×). The dermatoscope facilitates visualization of subsurface skin structures located within the epidermis, dermoepidermal junction, and papillary dermis, which are otherwise invisible to the unaided eye. Colors and structures visible with dermoscopy are required for generating a correct diagnosis.

1.0 **Study Endpoints (if applicable)**

- Clinical assessment response after 4, 8 or 12 weeks. This will be assessed by a blinded study team member comparing clinical photographs and dermascopic images.
    - Clinical photographs measured by no response, partial resolution, complete resolution.
    - Dermoscopy images: no response, partial resolution, complete resolution using the guidelines below that indicate the presence of wart or mollusum.
        - Molluscum: Visualization of a central umbilication with polylobular, white to yellow, amorphous structures is typical. A peripheral crown of radiating or punctiform vessels is also present.
        - Wart: grouped papillae, with dotted or loop vessels, and/or hemorrhagic points and lines often surrounded by a whitish halo
- Visual analogue scale for experimental treatment and control treatment
    - The visual analogue scale (VAS) is a validated subjective measurement of pain experienced by the patient. It consists of visual-numeric scale, numbered from 0-10. (0) indicate absence of pain or “no pain at all”, while (10) indicates severe pain or “worst imaginable pain”. This is supplemented by six faces with different expressions, ranging from happy to extremely upset. Each facial expression is assigned a numerical scale from 0 to 10.
- Evaluation of tolerability included assessment of dry ness, peeling, scaling, erythema, edema, stinging, burning sensation, itching, scaring and dyspigmentation. They will be assessed by means of a standardized grading scale (0 = absent; 1 = mild; 2 = moderate; 3 = severe)

5.0 Inclusion and Exclusion Criteria/ Study Population

Inclusion Criteria

- All patients from 4-21 years old with at least 1 lesion of vercurra or molluscum.
- Willingness of the participant and their guardian to provide consent when applicable.

Exclusion Criteria

- Unwillingness to participate in the study
- Received any treatment on the lesion in the past month determined by review of their medical record
- Immunodeficiency determined by review of their medical record.
- Adverse response to prior treatments determined by review of medical record.
- Signs of self-resolution determined by study team members.
- Conditions that lead to excessive scarring determined by study team members.
- Face and genital lesions determined by study team members.

6.0 Number of Subjects

Total of 150 would be required (half control, half NTP) to achieve 80% power with an alpha of .05.

Calculation:

- Assumption for Probabilities for the Null hypotheses is P1=P2=P3=1, hence P1 = .333333, P2 = .333333, P3 = .333333 and P1+P2+P3 = 1.0 or that each proportion has an equal chance.
- Assumption for Probabilities for Alternative Hypothesis is taken from Provided NTP paper where P1= 9/17 (Full Resolution = .53), P2 = 3/17 = .18 (Some Improvement), and P3 = 5/17 =.29 (no improvement).

7.0 Setting

MUSC dermatology clinics

8.0 Recruitment Methods

Recruitment will occur during standard of care visits to the Dermatology and Pediatric Dermatology Clinics, virtual visits, and chart review of eligible patients.

For standard of care visits, someone directly involved in the participants patient care will assess the patient's eligibility and inform eligible patients and/or their legal guardian about this study. If they are interested, the study team members will approach the patients or their legal guardian without coercion and with the emphasis on the voluntary aspect of being on this study. Subjects will also be told in a caring manner that no matter what their decision is, it will not affect how their doctor cares for them as a patient or their care in general.

Potential subjects who contact the study team members directly will also be considered for participation in the study. In this event, these potential subjects must have heard about the study through indirect information sources (clinicaltrials.gov or other means by which the study team members did not directly advertise the study for purposes of recruitment). If they

are interested, the study team members will respond to them via email, and subsequently approach the patients virtually without coercion and with the emphasis on the voluntary aspect of being on this study. Subjects will also be told in a caring manner that no matter what their decision is, it will not affect how their doctor cares for them as a patient or their care in general.

9.0 Consent Process

Potential subjects and/or their legal guardians will be approached by researchers at MUSC Dermatology and Pediatric Dermatology clinics. Members of the team will approach their own patients to ask for participation. Pertinent project information, risks and time commitment will be relayed to subjects. If subjects show interest in participating, they will be given an assent form to either sign or bring home for consideration.

Minimally, an adult parent with legal custody of a child or an adult awarded legal custody of a child must give informed consent for a child to be enrolled in research. A child 12 years of age or older must give documented "assent" to be enrolled in research. The assent will be documented on the informed consent document. An "emancipated minor" must provide documentation of his/her financial independence such as a rental lease, marriage certificate or court document in his/her name proving emancipation before consenting as an adult to participate in research. If child reaches 18, while enrolled on the study they will be reconsented without parents. All patients 18 years and older will be consented as adults.

The consent process will occur in a private room; the research team will explain to the participant and/or their legal guardian a language they can understand what it means to take part and what they can expect. Research team members will be obtaining assent when applicable. The research team will use language and terminology that will be understood by participant. A device demonstration will be showed to the child and their parents. The child and parents will be encouraged to ask questions at the end and sign their understanding. No waiting period will be necessary between informing the subjects and obtaining the consent however, subjects will be allowed to take home the unsigned consent form for review prior to signing it if needed and reschedule another visit for treatment as soon as possible.

10.0 Study Design / Methods

- A chart review of patient the patient's medical record will be completed and the following information will be collected for research purposes: DOB, demographics, contact information and diagnosis.
- Skin irritants, emollients, topical medications and other topical products cannot be applied to the study area 4 weeks prior to the start of the study and throughout the duration of the study. Details of what can and cannot be used can be discussed any time.
- During a clinical care visit, if a patient is eligible and agrees to participate, lesions will be selected by the dermatologist prior to procedure. The lesions will be marked randomly for a certain treatment regimen and photographs will be taken before each treatment and after completion of the study. Identifiers will be covered to protect identity. All images will be saved on a MUSC network secure box drive using a subject study number.
- The number of lesions treated will not exceed SOC recommendations. They will be determined by provider assessment and anatomical location.
- Participants with one lesion will receive either NTAP or standard of care (SOC). SOC is

cryotherapy for warts and cantharidin for molluscum. Participants with greater than 2 lesions will receive equal treatment of NTAP and SOC. The number of lesions treated will be based off the providers assessment and anatomical location. Treatment of a single lesion or an odd number of multiple lesions will be based off a randomized list.

- All SOC lesions will be treated first to reduce the number of patients not wanting return to the more painful modality.
- All people in the room will be provided eye shields that must be worn during treatment. The patient will be placed in a comfortable position before the lesion is cleaned with alcohol.
- Each lesion will be treated for 1-2 minutes. Total treatment duration will be no longer than 20 minutes.
- The interval between treatment groups will about 1 minute.
- There will be a follow-up phone call a week after treatment to check on resolution and report any adverse events. If an adverse event is reported the study team members will access if the patient needs to be seen by a provider that day.
- Patient will return to the office for a 4-week standard of care follow-up. Study cessation or additional treatment will be determined by the provider assessment of lesion clearance. If lesion persist another treatment will occur. No greater than 3 treatments will be performed.
- This study will be single blinded, meaning the study team member accessing the results will not know which lesions were treated with what therapy. Assessment of clearance will be based off of clinical examination and images. The team will record any additional clinical feature that may arise during the analyses.

11. Data Management

- Continuous data will be summarized using descriptive statistics (number of values, means, standard deviation, median, minimum and maximum). Categorical data will be summarized using frequency tables (frequencies and percent).
- Chi-square analysis comparing the proportions of successes (equal chance outcomes).
- Coded dermoscopic and clinical photographs may be obtained for further analyses after each visit. Files with patient images will be stored in a secure departmental drive that will only be accessible to study team members by their unique MUSC ID and password.
- All subject medical record information and data collection will be coded and stored on an MUSC Box drive only accessible to study team members and will be protected by two-factor authentication. The linking document between ID and PHI will be maintained separately from the study data in a separate encrypted folder.

14.0 Withdrawal of Subjects (if applicable)

- Patient may withdraw at any time. Subjects may verbally notify investigators if they wish to voluntarily withdrawal from the study immediately. All data collected until this point will be retained for study purposes.

15.0 Risks to Subjects

Risk for NTAP: To date, there are no known side effects or risks associated with the use of cold atmospheric or studies producing discomfort or pain (20-26, 29). However, because this is an experimental device, side effects like blistering, scarring, significant pigmentary alteration, and/or persistent nail change could occur and will be monitored at each visit, as well as pain assessment at every treatment session by validate pain scale (VAS).

The treatment received may prove to be less effective or to have more side effects than the other study treatment(s) or other available treatments.

This is a new device and even though no side effects of this treatment are reported in literature to date (27), the IRB will be notified via amendment, as well as participants via phone call if one arises.

There is a risk for possible breach of confidentiality. However, all measures to keep information protected will be taken. Photographs will be stored in the coded study-specific medical record for further analysis of lesion features. Only study team members will be able to access.

A cumulative data assessment will be conducted every other month throughout the study's duration by study team members. A report that includes any available efficacy data, as well as a record of anticipated adverse events, can be compiled and submitted to the IRB at that time. Any event meeting the criteria of an unanticipated problem involving risks to subjects or others will be immediately reported to the MUSC IRB, as required by HRPP 4.7- Unanticipated Problems and Adverse Events Policy and Procedures.

The plan for subject safety and minimizing risks of the research is assessing AE during, immediately following treatment and a few days after. Participants are encouraged to reach out to the study team if they think they are experiencing any adverse events. Other expected adverse effects deemed intolerable by the patient will prompt reduction in the patient's frequency, duration and treatment if indicated. Any condition necessitating cessation of the study drug will be followed to resolution.

16.0 Potential Benefits to Subjects or Others

Improvement may be seen in Molluscum Contagiosum and Verruca Vulgaris from participating in the study. However, there is no guarantee. Participation may help other patients in the future by increasing awareness and making it more readily available.

17.0 Sharing of Results with Subjects

Treatment outcome will be shared with subjects upon written request.

18.0) Drugs or Devices (if applicable)

- NTAP device will be stored in a locked office, handled by trained study team members and used only on subjects enrolled in the trial.
- Investigators will be in charge to handle dermoscope and will be responsible to upload the images to the secured folder. The Dermatoscope is device used SOC for analysis of DSAP lesions.

- We are applying for the device to be reviewed as an NSR device. Dr. Friedeman received NSR device approval at his institution for a similar study (6).

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