PROTOCOL

NANO-PULSE STIMULATION IN SEBACEOUS HYPERPLASIA

(NP-SH-006)

MAY 22, 2018

CONFIDENTIAL

PMB/5/22/18

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BACKGROUND

Sebaceous Hyperplasia (SH) is a common condition that appears as white or lightly pigmented bumps or bulges on the skin that occur when hyperactive sebaceous glands produce excess oil that pushes up on the skin surface. There are sebaceous glands all over the body, so the SH bumps can form almost anywhere, though there are more frequently observed and treated when they appear on facial skin. These benign lesions SH are more likely to occur in middle-aged and older people, but they can show up at any age. The sebum produced by sebaceous glands is excreted through skin pores and helps the skin form a lipid barrier to common environmental stresses. Too much sebum production can contribute to several problems, including acne, oily skin, or an oily scalp. When Sebum can also clog a pore and create and cause selling between the gland and the surface of the skin, causing it to swell and form a bump under the skin. Some medications and home remedies may reduce the appearance of bumps or reduce sebum production, and many patients request cosmetic procedures to remove them. There is currently no permanent cure for sebaceous hyperplasia.

Several factors increase the likelihood of developing sebaceous hyperplasia. Fair-skinned people older than 40 tend to develop the condition, especially when their skin has frequently been exposed to the sun. Continual sun damage can worsen symptoms or cause them to appear earlier. People may be more likely to develop sebaceous hyperplasia if they have a family history. People with suppressed immune systems and those taking the immunosuppressant medication cyclosporine may have a higher risk of developing sebaceous hyperplasia.

The main symptom of sebaceous hyperplasia is the appearance of small, shiny bumps under the skin. A bump can have a slight indentation in the center and a white or yellow outer edge. It may be difficult for a non-dermatologist to distinguish the condition from acne. However, a whitehead or blackhead will usually have a lifted center, while bumps caused by sebaceous hyperplasia are indented. These SH bumps are typically small and cause no pain. Many people with oily or combination skin may notice these bumps as they age. Bumps may appear on their own or in small clusters.

A sebaceous hyperplasia lesion is benign, and does not usually require medical treatment. However, many patients choose to have them removed for cosmetic reasons. if the bumps are unsightly or embarrassing. Various modalities are available to improve the appearance of SH lesion, but a few sessions or applications are often required for full removal and prevention of recurrence. Remedies include topical Retinol, facial peels, laser therapy, cryotherapy, electrotherapy, photodynamic therapy, excisional surgery, antiandrogen medications, and a variety of home/over-the-counter remedies. Pulse Biosciences proposes to conduct a clinical evaluation of their Nano-Pulse Stimulation

(NPS) device as a modality in the treatment and management of Sebaceous Hyperplasia (SH) in patients bothered by their appearance.

NPS TECHNOLOGY REVIEW

The NPS medical device is intended to clear the skin of benign, undesired skin lesions as an alternative to surgery and other more destructive methods for removing benign lesions. The system is designed to deliver a timed series of very low energy, high voltage (about the same as the voltage of static electricity) pulses of a time length between 100 and 750 nanoseconds (billionths) of a second). The non-thermal effect on tissue takes place in a very shallow depth of skin directly below the sterile treatment tip. The device emits significantly less thermal energy than existing laser, electro-surgery or electro-cautery equipment.

Extensive *in vitro* research demonstrates stimulation of a form of delayed programmed cell death in a treated area, which is one of the identified mechanisms for observations of clinical and histologic changes to cells in multiple studies of *in-vivo* treated tissue in both humans and animals. In pre-clinical studies with over 1000 rats and mice, nano-pulsed devices have been demonstrated to reduce or eliminate malignant tumors with a clinically acceptable margin of safety. A pilot clinical trial of common skin cancer lesions was conducted under IRB oversight in 2012 with a similar version of the NPS device, with the findings published in *Experimental Dermatology.* A total of ten basal cell carcinoma lesions on the skin of three Subjects were treated with a range of settings. Seven of the ten lesions were free of basal cells at the conclusion of the study, two partially resolved, and one recurred. No scars were visible at the healed sites of any of the successfully treated lesions.

PRIOR NPS CLINICAL STUDIES

The NPS device has been successfully deployed in several clinical studies of skin and subcutaneous structures. As of the date of this writing, the following studies have been successfully conducted and concluded: 1) A histological study evaluating use of the NPS on healthy abdominal human tissue in a dose-response study; 2) A histological study evaluating use of the NPS and comparative devices on healthy abdominal human tissue, 3) histological study following use of the NPS on healthy facial skin tissue in the pre-auricular area; 4) A multi-center study in the treatment of Seborrheic Keratosis lesions Additional detailed summaries of these studies follows.

Abdominal Tissue (Pre Abdominoplasty) Studies

Study Name: Nano-Pulse Human Tissue Study
Study Number: NP-ABD-001
Device Named: NPS
PI Name: David Kaufman, MD

Co PI: Michele Martinez, RN
Study Dates: November 2016

A total of eight Subjects participated in this prospective, single site, non-randomized study. All of the participants were females representing a wide range of Fitzpatrick Skin Tone Classifications, and ranging in age from the low 40's to middle 60's.

The study was comprised of two sequential segments. The first segment evaluated the serialized use of the test device in demarcated locations on intact abdominal skin predetermined for subsequent resection during a standard abdominoplasty scheduled at a later date. The primary endpoint of the first segment of the study was to establish that the trauma to the tested skin would be minimal over a range of six progressively increasing energy levels. The appearance of each intact tested tissue location was comparable to that anticipated following any minor surgical excisional procedure. A total of 210 NPS applications were tested and analyzed in this study.

Lidocaine was injected prior to use of the NPS skin exposure. Subjects reported little or no sensation during the treatment session. Subjects further reported little or no discomfort after the Lidocaine effect had diminished. There were no adverse events, no reports of bleeding, and no scarring noted by the investigator at the end of the 60 day study

The second segment of the study called for the histological analysis of tissue samples prepared from the abdominal section that were resected at the time of the abdominoplasty. Internal technicians and outside expert dermatopathologists evaluated the skin samples using standardized methods of microscopic analysis. This second segment did not impact the participating Subject as it occurred after the abdominoplasty procedure. Consequently, Pulse Biosciences, Inc. secured IRB oversight concentrated on the first segment of the study. It should be noted however that the histological analysis to date confirmed that the NPS was successful in achieving its intended effect across the range of tested energy settings.

Abdominal Tissue (Pre-Abdominoplasty) Comparative Studies

Study Name: Nano-Pulse Human Tissue Study
Study Number: NP-ABD-001C
Device Named: NPS
PI Name: David Kaufman, MD
Co PI: Michele Martinez, RN
Study Dates: August 2017

A total of 2 Subjects participated in this prospective, single site, non-randomized study. Both of the participants were females representing two different Fitzpatrick Skin Tone Classifications, and ranging from the high 20's to low 40's in age.

The primary purpose of the study was to collect tissue samples from healthy human

resected tissue that had been exposed to various methods of partial tissue ablation prior to a planned abdominoplasty resection date. The skin samples were taken from a section of abdominal skin that was previously exposed to the effect of the NPS during the same time period as two comparator medical devices considered to be the "gold standards" for tissue ablation. Histological analysis of the resected abdominoplasty tissue was performed on samples taken from sites treated with the 2 "gold standard" devices as well as the NPS.

The study is best described as comprised of two sequential segments. The first segment called for the serialized use of the NPS test device and the gold standard "comparatives" in specific locations on intact abdominal skin predetermined to be resected during a standard abdominoplasty. The impact of the study test device, the comparative devices and/or the study process ceased at the time of the abdominoplasty. The primary endpoint of the first segment of the study was to establish that the apparent trauma to the tested skin was minimal for all devices. The appearance of each intact tissue location that was tested with the NPS or the two comparative systems was comparable to that anticipated following any minor surgical excison procedure.

Lidocaine was injected prior to use of the NPS skin exposure, and one of the comparators. Subjects reported little or no sensation during the multiple treatment sessions. Subjects further reported little or no discomfort after the Lidocaine effect had diminished in the days following each treatment. There were no adverse events, no reports of bleeding, and no scarring in the NPS group noted by the investigator at the end of the 60 day study

The second segment of the study called for the histological analysis of tissue samples prepared from the abdominal section that was resected at the time of the abdominoplasty. Internal technicians and outside expert dermatopathologists evaluated the skin samples using standardized methods of microscopic analysis.

Pre-Auricular Tissue (Pre-Face Lift) Studies

Study Name: Nano-Pulse Pre Auricular Tissue Study
Study Number: NP-PT-003
Device Named: NPS
PI Name: James Newman, MD
Study Dates: October 2017

A total of 3 Subjects participated in this prospective, single site, non-randomized study. Two of the participants were females and one was a male representing varying different Fitzpatrick Skin Tone Classifications and all were over the age of 50 years of age.

The study was compromised of two sequential segments. The first segment called for the serialized use of the test device in several locations on intact facial skin pre-determined to be resected during a standard face-lift, which is typically a small section in front of the ear (the pre-auricular area).

The primary endpoint of the first segment of the study was to establish that the visually apparent trauma to the tested skin would be minimal and the appearance of each intact tested tissue location was comparable to the appearance that is anticipated following any minor surgical procedure.

Lidocaine was injected prior to NPS administration to the skin surface. After the first NPS exposure, Subjects returned at varying time intervals prior to their scheduled face lift procedure for additional NPS administrations on demarcated areas on each side of their face using varying tip sizes and energy settings. Subjects reported little or no sensation during the NPS administrations. Subjects further reported little or no discomfort after the local anesthetic effect had diminished. There were no adverse events, no reports of bleeding and no scarring noted by the Investigator at the end of the study.

As was noted for the pre-abdominoplasty tissue study the second segment of the pre face lift study called for the histological analysis of tissue sample prepared from the tissue section that was resected at the time of the face lift. Internal and outside expert histologists were consulted. See Attachment A for a report authored by Darius R. Mehregan, MD, and expert dermatopathologist, based on his independent analysis of the biopsy samples from this study of facial skin.

Following the same model as described above, additional studies were performed involving pre-auricular tissue of facial skin in 3 Subjects scheduled for eventual face lift procedures. The sole Investigator for this series (NP-PT-003) was Dr. James Newman, MD., Plastic Surgeon, in San Mateo, CA. As noted above for the pre Abdominoplasty study, local anesthetic was administered prior to the NPS skin exposure. Subjects reported little or no sensation during the treatment session. Subjects further reported little or no discomfort after the local anesthetic effect had diminished. There were no adverse events, no reports of bleeding, and no scarring noted by the PI at the end of the 60 day study.

Seborrheic Keratosis Study

Study Name:	Nano-Pulse Seborrheic Keratosis Study
Study Number:	NP-SK-002
Device Name:	NPS
PI Names:	James Newman, MD George Hruza, MD Thomas Rohrer, MD Brian Zelickson, MD
Study Initiation Date:	May 2017

The SK Study was a prospective, randomized, open label, multi-site, NSR study design where each Subject served as his or her own control. Principle Investigators were identified at each of four participating sites. A total of 58 Subjects participated. Forty eight, 48 (83%) of the participants were females and 10 (17%) were male. The Subjects were primarily Caucasian (95%). Fifty one, 51 (88%) were classified as Fair or Medium using the Fitzpatrick Skin Tone Classifications, and ranged in age from 34 to 74 years old.

For each Subject, 4 SK's were selected that met the study criteria for treatable SK's. If more than four lesions were present, the Subject stated a preference for which 4 lesions would be included in the study. Photographs of each of the four selected SK's were taken prior to anesthesia or treatment. Local anesthesia was injected at the site of the four selected SK's. Using a pre-determined randomization method, three of the selected SK's were designated to be treated and one was designated as an untreated control. The test device was deployed to the selected SK's according to a randomized order. Subjects returned at prescribed intervals for evaluation of the treated lesion as well as the skin around the lesion. Photographs were taken of each lesion at all intervals.

Among the 58 Subjects, 58 control lesions were identified and 174 lesions were treated. Lidocaine was injected prior to the NPS skin exposure. Subjects were asked to rate their discomfort on a 0-10 point scale where 0=no discomfort. Subject's reported little or no sensation during the treatment session. Subjects further reported little or no discomfort after the local anesthetic effect had diminished. Ratings were analyzed on a per Subject basis and on a per lesion basis. 69% of Subjects rated their discomfort at None, Minimal or Mild and when considered on a per NPS administration basis, 68% reported discomfort to be None, Minimal or Mild.

Each lesion area was routinely examined at each study visit for the presence of bleeding, scabbing, crusting, erythema, swelling, exudate, indurations, etc. Evidence of blood

tinged oozing was noted at the pre-treatment site of some of the injected Lidocaine anesthesia infiltrations. No frank bleeding was noted at any time.
Minor scabbing, crusting and flaking were noted in the early post treatment visits but lessened over time in the natural course of skin healing. There were no adverse events and no complications.

At the final 106 day post treatment visit, using a standard scale, the investigators rated the clearance level of each lesions and the appearance of the residual skin. The Subjects rated their satisfaction with the appearance of each lesion. Investigators were asked to observe and rate the lesion clearance using the following scale: *0 = Clear; 1= Mostly Clear; 2= Partially Clear and 3= Not Clear*. Each treated lesion was rated at each 1-month post; 60 days post; 90 days post and 106 days post treatment. The treated lesion was considered improved when at least partial clearance was observed. Combining all three clearance levels, 87% of the lesions had improved at 1 month and 93% showed improvement at 106 days post treatment.

Investigators used a comparable scale to evaluate and report the appearance of the skin within the margins of the NPS location and adjacent to each lesion location. The scale provided included: 0 = *Clear; 1 = Hyper-pigmentation; 2 = Hypo-pigmentation or 3 = Other* as rating options. 32% of the surrounding skin sites were recorded as *Clear.*

Some *Hyper-pigmentation* was noted for 60% of the treated lesions surrounding skin. *Hypo-pigmentation* was seen in 2% and 6% of the ratings were recorded as *Other*.

Subjects were asked to rate their level of satisfaction with the outcome for each of their treated lesion. The rating scale included: *Satisfied; Mostly Satisfied, Partially Satisfied, Dissatisfied and Highly Dissatisfied*. Subjects reported being at least partially Satisfied for 93%, (n=161) of the treated lesions.

An Independent Photographic Review was conducted following the close of the study. Each reviewer rated photographs of 196 lesion sets taken at the pre-treatment and 106 day visits respectively. This review was conducted in accordance with established standards for validity and reliability. The rating scale of the independent review was the same one utilized by the Principle Investigators who participated in the study. Prior to its use in the Independent Photographic Review, the Rating Scale was validated. Inter-rater Reliability was established at 82% and Intra-rater Reliability was established at 83%. Success Criteria for purposes of the Independent Photographic Review was set as an improvement of at least 2 grades on a 4 point rating scale. 71% of NPSs met the success criteria.

PRIOR CLINICAL STUDIES CONCLUSIONS

A combined total of 642 NPS applications have been delivered to 71 adult Subjects. Anatomic locations included abdomens, backs, arms, legs and faces. Discomfort was managed with localized Lidocaine. For 58 of the Subjects a favorable therapeutic outcome was established in a significant majority. Side effects consisted of relatively

minor reactions consistent with routine wound healing. No complications or adverse events were reported.

PROPOSED SEBACEOUS HYPERPLASIA STUDY

Study Design

Sebaceous Hyperplasia (SH) is a common condition that appears as white or lightly pigmented bumps or bulges on the skin that occur when hyperactive sebaceous glands produce excess oil (sebum) that pushes up on the skin surface. These benign SH lesions are more likely to occur in middle-aged and older people, but they can show up at any age. There is currently no permanent cure for sebaceous hyperplasia.

A sebaceous hyperplasia lesion is benign and painless, and does not usually require medical treatment. However, many patients choose to have them removed by physicians for cosmetic reasons, especially if the bumps are viewed as unsightly or embarrassing. . Various modalities are available to improve the appearance of SH lesions, but a few sessions or multiple applications are often required for full removal and prevention of rapid recurrence. Remedies include topical Retinol, facial peels, laser therapy, cryotherapy, electrotherapy, photodynamic therapy, excisional surgery, antiandrogen medications, and a variety of home/over-the-counter remedies.

The proposed SH Study using NPS treatment of individual lesions is a prospective, open label, multi-center non-significant risk study design where each Subject serves as his or her own control. A Principle Investigator (PI) will be identified at up to 4 participating study centers. A total of up to 75 Subjects will be consented and enrolled with an anticipated enrollment expected to be uniformly distributed across sites. The PI will consider the study inclusion and exclusion criteria and invite appropriate patients to participate in the study.

The intended treatment device is described as the Nano-Pulse Stimulation (NPS) device and has been used in a similar manner in prior studies of skin lesion clearance. For the proposed SH lesion study, the skin contacting device component referred to as the “tip” will be generally be 1.5mm x1.5mm or 2.5 mm x 2.5mm in size with energy settings in the mid-range of previously tested energy levels. Local anesthetic in the treatment area will be used as needed to manage potential treatment discomfort.

Each Subject may present with a minimum of 2 and up to 4 designated SH lesions, with one lesion designated as an untreated control by a randomized method. All treated lesions will receive at least one NPS treatment. At 30 days post-treatment, if any treated lesion that is rated by the investigator as *Not Clear* or *Partially Clear* on a study standardized scale will be eligible for a second treatment to enhance lesion clearing. If a second treatment is indicated for some or all of the lesions the second treatment will occur at 30 days after the initial treatment. Total study time for these Subjects with any lesion requiring two treatments will be 90 days. Subjects with all treated lesions requiring only one treatment will complete the study in 60 days.

No significant risk is posed by either the use of the NPS or the study process. While safety can never be taken lightly, this study is not meant to measure monitor or analyze any significant disease or disorder for which medical treatment is mandatory.

See additional information regarding Non-Significant Risk statement below.

Non-Significant Risk Statement

The proposed study fits the criteria for a non-significant designation for the following reasons:

- Subjects will be recruited to participate in the study on a voluntary basis. Potential participants will be recruited directly from the Investigator's population of patients who are candidates for removal of their SH lesions.
- Participation in the study takes no more than approximately 1 hour for each of the maximum of 5 study visits.
- Localized anesthesia will be used to control discomfort at the time of any NPS device applications.
- After the NPS device is deployed and the anesthesia is dissipated, there may be mild, localized discomfort at the site of the treatment. A small scab or crust may develop. This occurrence is transient and should resolve without intervention.
- Prior studies support the proposal that the NPS use represents minimal risk.
- There is no loss of privacy as no study records will be viewed or retrieved by anyone other than the study team members.
- Protected information will not be captured.
- Subject identity is protected by a coding system to de-identify them.

Study Objectives

- Per SH Lesion: NPS treated lesions will be rated via a previously used scale for similar NPS studies. At the Final Study Visit, the PI will rate each lesion in comparison to an image of that same, pre-treated lesion. At least 50% of the NPS treated lesions will be rated as "CLEAR" or "MOSTLY CLEAR".
- Per Subject: At the Final Study Visit at least 50% of the Subjects will present with 50% of their lesions rated as "CLEAR" or "MOSTLY CLEAR".
- No AE's or complications will be reported specific to the NPS device or the study process.

Success/Failure Criteria

Study outcome measures will be applied at every study visit and success/failure criteria will be applied at 60 days after the first NPS treatment for lesions treated one time or 90 days after the first NPS treatment for those lesions treated twice. Success will be determined as described in the preceding paragraph regarding Study Objectives. In patients with both single treatment lesions and multiple treatment lesions, all treated lesions will be evaluated and images captured at all visits, regardless of number of treatments

Investigator/Site Selection
Investigators/Site selection and qualification will be initiated upon IRB approval of the protocol and consent provided in this submission. Several of the potential PIs have participated in prior studies using the NPS device.

Subject Selection
Up to 75 Subjects with 2-5 qualifying SH lesions who are interested in removal of those SH lesions and meet the full study criteria will be consented to participate in a study to evaluate the outcome using the NPS device. The specific study Inclusion and Exclusion criteria are outlined in the following paragraphs.

Inclusion Criteria: (Must be a "YES" Response)

- Willing to sign the Informed Study Consent
- English speaking/reading and/or translation is available for all visits
- Males or females
- At least 18 years of age but less than 71 years of age
- Presents with at least 2 and up to 5 clinically visible SH lesions
- Understands that 1 lesion will remain untreated to act as a reference
- Lesions must measure no greater than 2.5 x 2.5 at the outside margin
- Wishes to have at least 1 and perhaps 2 NPS treatments to each study lesion
- Selection of the non-treated reference lesion will be randomly identfied
- Willing to return to the PI's office for 4 or 5 total study visits at specified intervals over 60 or 90 days
- Agrees to photographic or other image capture methods of both the treated and untreated lesions.
- Agrees to avoid any other treatment to the NPS treated SH lesions and to the untreated SH lesion until the end of the NPS study
- Understands that their personal identity will be masked and all availability of images will be restricted to the study team.
- Has no evidence of active infection in the designated tissue prior to treatment and reports no infection within 90 days
- Is not allergic to Lidocaine or Lidocaine-like products
- Investigator determines that the patient is in good general health and is unlikely to be subject to risks from the treatment

Exclusion Criteria: (Must be "NO" Responses)

- Presence of Implantable electronic devices that cannot be removed. e.g., pacemaker or automatic defibrillator
- Taking medications prescribed for cardiac arrhythmia at any time within 6 months prior to exposure to the NPS device
- SH lesions are located within the eye orbit or on the nose

- Active infection or history of infection in designated test area within 90 days prior to study initiation
- Use of oral steroid and/or retinoid use within the last 12 months
- Prior physician ordered treatment to the identified SH lesions targeted for the study which occurred within 6 months prior to study start
- Is known to be immune-compromised and/or received immunosuppressant therapy within 6 months prior to study start
- Taking blood thinning medications
- Has Insulin dependent diabetes
- Is known to be pregnant or lactating female

STUDY PROCESS

The following is an example of the Subject related activities to be completed for each Subject in the study. The exact order may differ depending on Subject/PI schedules and preferences.

Activities prior to or on same day as study enrollment and occur on same day as treatment visit:

- Evaluation for Inclusion/Exclusion criteria
- Sign the consent form prior to any study activities
- Receive a copy of the signed consent form

Activities on 1st Study Visit Day-NPS Treatment Day

- Up to 5 SH lesions that meet the study criteria are selected for inclusion in the study. Specifically….
- if 2 SH lesions are qualified, 1 will be treated and 1 will be reference
- If 3 SH lesions are qualified, 2 will be treated and 1 will be reference
- if 4 SH lesions are qualified, 3 will be treated and 1 will be reference
- if 5 SH lesions are qualified, 4 will be treated and 1 will be reference
- Using a randomization method the selected SH lesions will be designated to be treated and one will be designated as an untreated control
- Images of each of the selected lesions will be taken prior to anesthesia or treatment
- Local anesthesia will be applied to all study selected SH lesions
- The lesions to be entered into the study will be numerically labeled. Those lesions to be treated vs the reference lesion (non-treated) will be randomly selected.
- A light bandage and any PI recommended dressing will be applied
- Subject will be discharged

Activities on 2nd Study Visit Day – 5 Days post NPS treatment

- Images of each of the treated lesions and one non-treated lesion will be taken
- Each lesion will be clinically assessed and the appearance will be rated according to specified criteria.
- Any adverse events will be identified and documented.

Activities on 3rd Study Visit Day – 30 Days post NPS treatment

- Images of each of the treated lesions and one non-treated lesion will be taken
- Each lesion will be clinically assessed and the appearance will be rated according to specified criteria
- If any lesions are rated other than *Clear* or *Mostly Clear,* a 2nd NPS treatment may be delivered to that previously treated lesion
- Any adverse events will be identified and documented

Activities on 4th Study Visit Day – 60 Days post NPS treatment #1

- Images of each of the treated lesions and one non-treated lesion will be taken
- This is Final Study visit for any patient for whom all lesions were treated one time
- Each lesion will be clinically assessed and the appearance will be rated according to specified criteria
- Any adverse events will be identified and documented

Activities on 5th Study Visit Day – 90 Days post NPS treatment #1

- Images of each of the treated SH lesions and one non-treated SH lesion will be taken
- This is Final Study visit for any treated lesion
- Each lesion will be clinically assessed and the appearance will be rated according to specified criteria
- Any adverse events will be identified and documented

SH Study Follow-up Visit Schedule

Study Visit Interval	#1 NPS TX*	#2 5 days post NPS TX	#3 30 days post NPS TX	#4 60 days post NPS TX #1	#5 90 days post NPS TX
Study Visit Description	TX #1	Wound Check	Wound Check TX #2 (if applicable)	Wound Check Final visit for all lesions treated only one time	Wound Check Final visit for all lesions treated more than one time
Visit Range (# of Days Post First NPS TX)		4-8 days	28-35 days	57-67 days	87-97 days

*TX = Treatment

SUBJECT COMPENSATION

Each Subject will be compensated for any inconvenience that study participation may represent. Specifically, each Subject will make 4-5 total visits to the PI's office (approximately 4-5 hrs. total time). In return for this time commitment, the Subject will receive $200 per treatment visit, $100 per check-up visit and $200 for the Final Visit. An additional $400 bonus will be paid for completing all of the scheduled visits. The total amount of compensation will be up to $1000 to those Subjects who receive one NPS treatment and $1200 to those Subjects who receive more than one NPS treatment.

STUDY BENEFITS

A volunteer for the proposed study is someone who has elected removal of SH lesions. Such scars are typically removed via surgical excision, intra-scar steroidal injections or systemic steroids. Participation in the study offers another technique to accomplish the same thing as the typical tools. Experience with the NPS to date offers some confidence that the scars may be lessened although no assurances will be offered. The information learned may contribute to the ultimate use of a more safe and effective device available to treat benign and non-benign lesions in future patients.

STUDY/NPS DEVICE RISKS

The Investigators for the study are experienced physicians. Use of the NPS poses very minimal risk, including scabbing, minor skin pigment changes or scarring and minor discomfort which can be further minimized by covering the spot with a small bandage.

Typical wound complications such as infection, bleeding and discomfort which requires prescription medication for relief are not anticipated. If they should occur they would be considered an unanticipated adverse event and managed as such. If the Subject has any questions or concerns about their medical condition or if an unforeseen event should occur the Subject will be advised to contact the PIs or their designate.

SITE TRAINING PLANS

Training to be conducted can be divided into two pertinent categories. First is training on use of the NPS from a technical and clinical perspective. The PIs and their designated assistants will be instructed in aspects of set up, application,management and maintenance of the NPS. Those persons conducting the training will be Pulse Bioscience technical experts in this area of expertise. A select group of these experts will be present during the testing sessions to provide support as needed. Training will include a dry run. The second category is training in the clinical study requirements and processes. The study team members at the Study Site will be trained via the pertinent documents and files as well as the planned logistics to perform the study according to the protocol and applicable IRS, GCP and Pulse requirements.

INSTITUTIONAL REVIEW BOARD OVERSIGHT

The following Institutional Review Board will be asked to review and approve the proposed study:

Biomedical Research Institute of America

P.O. Box 600870

San Diego, CA 92160

T - 619 282 9997

F - 619 282 9998

If the Subject has any questions or concerns about the study process, they will be advised to contact Biomed at 619-282-9997

STUDY MANAGEMENT AND MONITORING PLAN

Pulse Biosciences, and/or their designated representatives are solely responsible for the proposed study. Pulse will take the necessary steps to assure that the study is conducted in accordance with all regulating authorities as well as applicable Standard Operating Procedures. The Study Monitor will oversee the conduct of the study on a continual basis. Minimal Monitoring visits will be conducted at the following intervals: Site Qualification Visit, Study Initiation Visit, Interim Study Visit and Study Close-Out Visit.

Attachment A

A Summary Histology Report of the Nano-Pulse Preauricular Facial Skin Study (NP-PT-003)

in a Pre-Excised Human Facial Tissue Model

Darius R. Mehregan, MD

Microscopic samples of preauricular facial tissue from three Subjects treated with a novel non-thermal energy device that delivers short low energy, high voltage pulses to a controlled tissue volume have been reviewed in a detailed histologic analysis. The treatment intervals of the targeted normal preauricular facial tissue ranged from 15 to 60 days prior to a previously scheduled facelift procedure in which the treated tissue was demarcated two months prior to excision. Six treatment energy levels are reviewed per patient and are evaluated at one of the three timepoints prior to their surgery: 60 days, 30 says and 15days. Various tissue staining techniques intended to identify specific cellular changes and tissue morphology were utilized in the histologic analysis to characterize the tissue responses and the subsequent recovery processes. The findings for each of the energy levels tested were compared to normal control punch biopsies in the same patient.

Epidermal Changes: Review of the changes of the entire thickness of the epidermal/dermal layer and subcutaneous fat from day 15 through day 60 was performed. The primary changes due to the non-thermal energy exposure were observed in the treated skin within the epidermal layer of skin.

In many patients, hair follicles and eccrine glands within the dermal layer of the skin were also visible for histologic review. In specimens in which hair follicles and eccrine ducts were visible, there was no apparent effect to the adnexal structures in any of the samples observed. This is consistent with previous histologic analysis of tissue exposed to this technology, which showed partial healing seen at 5 days and complete recovery by 15 days. Eccrine ducts often showed focal squamous metaplasia, a sign of re-epithelialization. The epidermal layer had returned to normal in all cases with a normal thickness and preservation of rete ridge pattern similar to controls.

The samples at the day 15 timepoints showed full re-epithelialization of the epidermis. The rete ridge patterns which often show flattening with chronic photoaging, also showed patterns similar to control specimens. Evidence of epidermal inflammation such as spongiosis or exocytosis of lymphocytes was not observed.

The samples at the day 30 timepoints showed similar changes to the 15-day samples.

The samples at the 60 days timepoint showed the epidermis, hair follicles, and eccrine glands had all completely returned to normal.

The epidermal thickness and rete ridge pattern was similar to control specimens. Distribution of eccrine glands and ducts as well as hair follicles were comparable to control samples. PT-002 showed evidence of follicular epithelial spongiosis and peri-follicular inflammation at day 15 which resolved by day 30.

Alterations of dermal collagen: Alterations in the dermal collagen observed were minimal, with no evidence of thermal injury. In some tissue samples exposed to treatment levels TL25.008, TL25.010 and TL25.012 there was a mild effect on the elastin fibers, as there was evidence of a loss of elastic fibers in the papillary dermis. In one of these patients (PT-003) there is also some flattening of the dermo-epidermal junction which improved by day 60.

Elastic tissue staining: Elastic tissue remained intact in the vast majority of patients. In tissue samples exposed to treatment levels TL25.008, TL25.010 and TL25.011 there was occasional slight decrease in elastic fibers. Minimal effects were noted on the dermis. This predicts a very low risk of scarring. The risk is greatest in treatment level TL25 011 and 012 (Evident in PT-003 30 days).

Melanocytic density: The number of melanocytes was observed using a MITF immunostain. The number of melanocytes is counted in 3 different areas of 1mm and averaged. Staining for melanocytes shows the number of melanocytes return to normal density, comparable to the control specimens for most energy levels tested. The two highest energy levels show a slightly lower count of melanocytes compared to controls, which may be indicative of a slower recovery of melanocytes. The rapid return of the melanocytic density to levels comparable to control should be consistent with a relative normalization of skin pigmentation over time.

Inflammation: Overall, the degree of dermal inflammation was minimal compared to other thermal or physical methods of intentionally damaging surface epidermal tissue. There is a small amount of inflammation seen at day 30 for some tissue exposed to treatment levels TL25.008, TL25.010 and TL25.012, however the amount of inflammation appears to be sparse, primarily perivascular and focally perifollicular. The samples examined showed no evidence of fibrin deposition in the blood vessels to suggest vascular injury.

Presence of Macrophages: Macrophages containing melanin were sparse but present in the papillary dermis. These were present at all treatment levels but are few in numbers.

Fat: There was no effect on the subcutaneous fat at any of the timepoints evaluated.

Summary: The novel method of using low energy, high voltage nano-pulse stimulation on facial skin and subcutaneous tissue at nine different energy settings was observed to lead to a predictable recovery of the epidermal layer of facial skin at all energy levels at all timepoints observed. The lack of observed effect on dermal collagen suggest that the effect is non-thermal, with a relatively low level of inflammation. This lack of inflammatory effect is consistent with preservation of fibroblasts, elastic tissue, and melanocyte recovery. The transient effect on deeper cellular structures in the dermis suggest an affinity of Nano-pulse energy for highly cellular tissue, and a sparing effect on the less cellular connective tissue of the collagen layer.

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