LED-Red Light Phototherapy for Skin Scarring Prevention

Principal Investigator: Jared Jagdeo, MD, MS

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STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP), applicable United States (US) Code of Federal Regulations (CFR), and the National Institute of General Medical Sciences (NIGMS) Terms and Conditions of Award. The Principal Investigator (PI) will assure that no deviation from, or changes to the protocol will take place without prior agreement from the funding agency and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

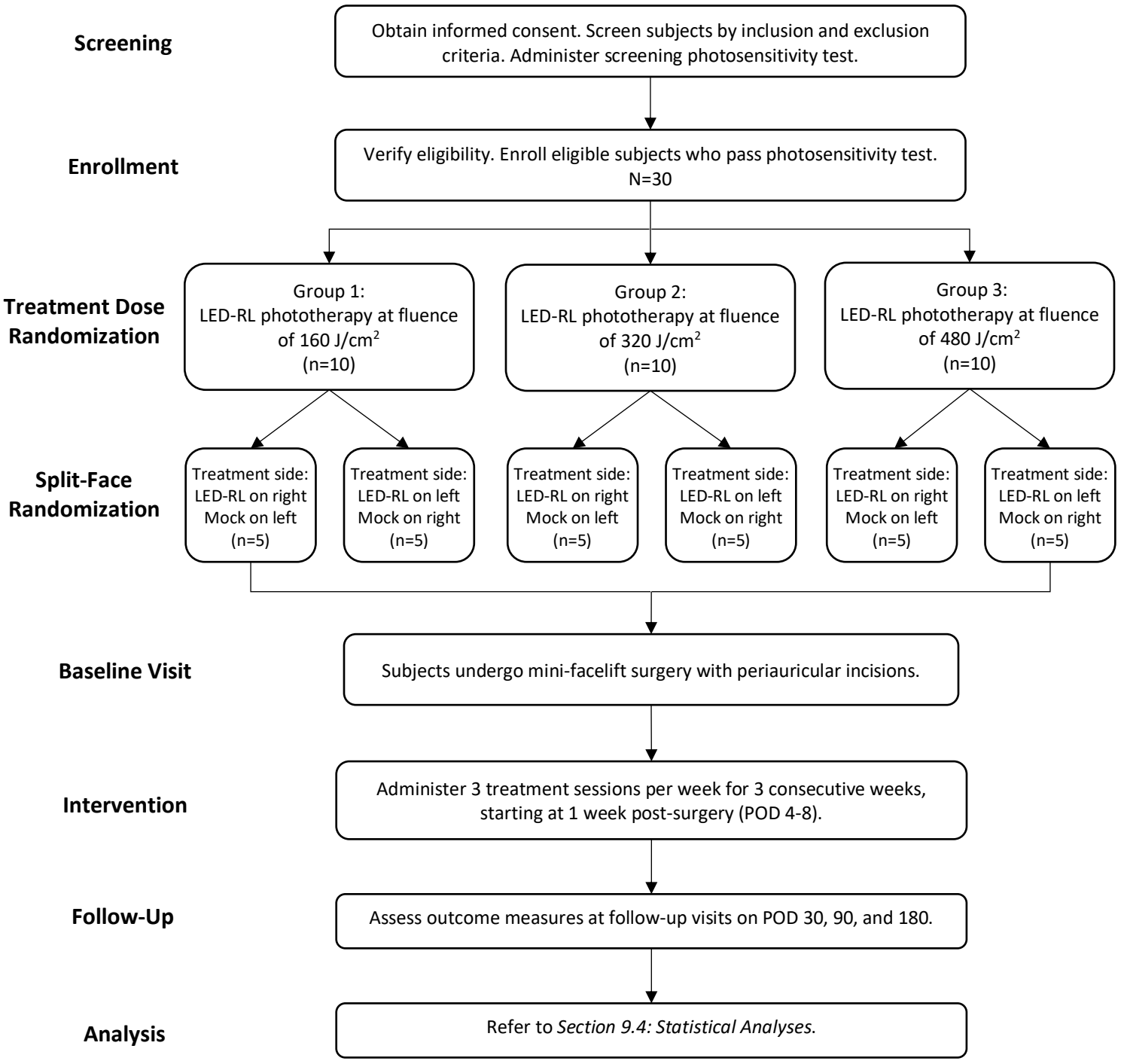
1.1 SYNOPSIS

Title:	LED-Red Light Phototherapy for Skin Scarring Prevention
Study Description:	This study will evaluate the safety and clinical efficacy of light emitting diode-red light (LED-RL) for the treatment of skin fibrosis in subjects who have decided to undergo elective minimal incision facelift (mini-facelift) surgery. Based on our *in vitro* data and Phase I studies, we hypothesize that LED-RL phototherapy may be a safe and effective therapeutic modality to prevent or limit cutaneous scarring after surgery. Subjects will use commercially available, FDA-cleared LED-RL phototherapy devices for the treatment of periauricular incisions associated with the mini-facelift surgery. A total of 30 subjects, randomly allocated to 3 treatment groups in a 1:1:1 ratio, will receive LED-RL at different fluences (doses) that were established as safe and well tolerated in Phase I studies. Using a split-face study design, each side of the face will be randomized to receive either LED-RL phototherapy or mock irradiation, allowing each subject to serve as his or her own control. The treatment will be administered 3 times weekly for 3 weeks, starting at approximately 1 week post-surgery, for a total of 9 treatment sessions. Efficacy assessments will include skin elasticity and induration measurements, tissue histology, rater-blinded photographic evaluation, three-dimensional skin imaging analysis, collagen measurements, optical coherence tomography (OCT), and subjective clinical assessments. Safety and adverse events will be monitored.
Objectives:	Using the fluences established as safe from our Phase I studies of LED-RL on normal human skin and hypothesized to be anti-fibrotic doses based on *in*

	vitro data (160 J/cm^2 to 480 J/cm^2), we will conduct a dose-ranging, randomized, parallel group, split-face, single-blind, mock-controlled Phase II study to evaluate the efficacy of LED-RL phototherapy in preventing or limiting skin fibrosis in a mini-facelift surgical wound model. Primary Objective: To evaluate the clinical efficacy of LED-RL phototherapy in the prevention of skin fibrosis in subjects undergoing elective mini-facelift surgery Secondary Objectives: • To evaluate the histological, ultrastructural, and molecular changes that occur *in vivo* as a result of LED-RL phototherapy • To detect clinically meaningful changes in scar characteristics after LED-RL phototherapy • To continue to assess the safety of LED-RL phototherapy on human skin
Endpoints:	Primary Outcome Measure: Difference in quantitative scar pliability between the treated and control incision sites Secondary Outcome Measures: 1) Difference in objective measurements of key scar characteristics (collagen and water concentration, wrinkles, texture, diameter, area, volume of elevation, pores, pigmentation, vascularity) between the treated and control incision sites 2) Difference in the Patient and Observer Scar Assessment Scale (POSAS) scores between the treated and control incision sites 3) Difference in the photograph-based Visual Analogue Scale (VAS) scores between the treated and control incision sites 4) Histological and molecular analyses of treated and control skin specimens 5) Incidence of adverse events 6)
Study Population:	A total of 30 subjects will be enrolled. Individuals of any sex, race/ethnicity, and age who are suitable candidates for mini-facelift surgery and who do not meet exclusion criteria will be eligible for this study.
Phase:	2
Description of Sites/Facilities Enrolling Participants:	Subjects will be enrolled at a single site, SUNY Downstate Medical Center. The study is not intended to include sites outside of the US.

Description of Study Intervention:	The LED-RL source is the Omnilux new-U handheld phototherapy device (GlobalMed Technologies, Glen Ellen, CA), which is commercially available over-the-counter and FDA-cleared for the treatment of periorbital rhytides. The device has a 4.7 cm x 6.1 cm rectangular aperture of LED arrays and emits visible red light (633 nm $\pm$ 6 nm) at a power density of 360.2 W/m^2 at room temperature. Each subject will be randomly assigned to receive LED-RL phototherapy at doses of 160 J/cm^2, 320 J/cm^2, or 480 J/cm^2 to one side of the face, with the contralateral side receiving mock treatment.
Study Duration:	24 months
Participant Duration:	6 months

1.2 SCHEMA



POD: post-operative day

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Screening within 6 weeks prior to surgery	Enrollment at 24 hours after screening	Baseline/day of surgery (Visit 1)	Treatment #1 (Visit 2)	Treatment #2 (Visit 3)	Treatment #3 (Visit 4)	Treatment #4 (Visit 5)	Treatment #5 (Visit 6)	Treatment #6 (Visit 7)	Treatment #7 (Visit 8)	Treatment #8 (Visit 9)	Treatment #9 (Visit 10)	Follow-up at POD 30 ± 1 week (Visit 11)	Follow-up at POD 90 ± 1 week (Visit 12)	Final study visit at POD 180 ± 1 week (Visit 13)
Informed consent	X														
Demographics	X														
Medical history	X		X												
Concomitant medication review	X		X	X	X	X	X	X	X	X	X	X			
Targeted physical exam of skin	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Conduct screening photosensitivity test	X														
Evaluate for photosensitivity		X													
Adverse event review and evaluation		X		X	X	X	X	X	X	X	X	X	X	X	X
Randomization		X													
Mini-facelift surgery			X												
Administer study intervention (LED-RL and mock therapy)				X	X	X	X	X	X	X	X	X			
Digital photography			X	X	X	X	X	X	X	X	X	X	X	X	X
Skin biopsy (optional)			X										X		
OCT				X									X	X	X
Skin elasticity and induration measurements				X									X	X	X
Collagen and water concentration measurements				X									X	X	X
Skin imaging analysis				X									X	X	X
POSAS				X									X	X	X
Complete case report forms	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Exit questionnaire															X

Screening Visit (within 6 weeks prior to surgery)

- Obtain written informed consent.
- Verify that the potential subject has opted to undergo elective mini-facelift surgery.
- Review medical history by interview to determine eligibility based on inclusion/exclusion criteria.
- Review list of current medications to determine eligibility based on inclusion/exclusion criteria.

- Perform targeted physical examination of skin. For this study, potential subjects must have excess lax facial skin that is suitable for mini-facelift surgery, as determined by the surgeon's clinical assessment. Potential subjects also cannot have any open wounds, pre-existing scars, fibrotic skin disease, or other medical conditions affecting the periauricular skin (i.e., the site of the surgical incisions and of the proposed LED-RL treatment).
- Conduct a screening photosensitivity test. Potential subjects will be exposed to LED-RL for 20 minutes on the nondominant upper forearm (as recommended by the manufacturer in the Omnilux user guide) and evaluated either in clinic or remotely 24 hours later to assess for adverse reactions.

Enrollment (24 hours after screening)

- Evaluate for photosensitivity by examining the nondominant upper forearm. Potential subjects have the option to return to clinic for an in-person evaluation, or have a remote follow-up via a phone call. For the remote follow-up, the research team will call the subject and ask if he/she has experienced any adverse reactions as well as request a photograph of the treatment area. Criteria for photosensitivity include, but are not limited to: warmth, erythema, edema, blistering, rash, pain, or discomfort lasting more than 24 hours. If no adverse reactions occur, the potential subject is deemed "non-photosensitive" and thus eligible for participation in the study.
    - Definition of screen failure: Subjects who have provided consent but subsequently fail to meet eligibility criteria for participation in the study based on screening procedures
    - Definition of enrolled: Consented and screened, with eligibility verified
- Randomize subject to treatment dose and treatment side as described in *Section 6.3: Measures to Minimize Bias.*
- Schedule study visits, including the day of surgery and subsequent 9 treatment sessions, for participants who are eligible and available for the duration of the study.

Baseline Visit and Day of Surgery (Visit 1, POD 0)

- Verify inclusion and exclusion criteria, including review of medical history and concomitant medications.
- Obtain pre-surgery photographs.
- Proceed with mini-facelift surgery under tumescent local anesthesia, performed by a board-certified dermatologic surgeon (Dr. Daniel Siegel).
- Obtain optional 2 mm punch biopsies of normal periauricular skin, taken from the excess lax skin that is excised as part of the mini-facelift surgery.
- Obtain post-surgery photographs.
- Provide standard of care post-operative instructions.

Treatment Sessions:

The first treatment session will occur approximately one week after surgery, defined as POD 4 to POD 8, a validated intervention time point for skin scar reduction therapy.[1] Treatment sessions will take place three times weekly (for example: Monday, Wednesday, Friday) for 3 consecutive weeks, for a total of 9 treatment sessions. Refer to *Section 6: Study Intervention* for the treatment protocol.

The following is a sample schedule. The specific POD of the treatment sessions in relation to the study visit number may vary among subjects depending on their exact date of the surgery (POD 0). Please

refer to *Section 8.1: Efficacy Assessments* for a description of all objective and subjective measurements that will be collected for outcome measures.

Monday (Visit 2, POD 5)

- Obtain pre-treatment session photographs.
- Concomitant medication review: Verify that the subject is not taking any photosensitizing medications that would preclude treatment with LED-RL phototherapy.
- Obtain baseline data for efficacy assessments: skin elasticity and induration measurements, collagen and water concentration measurements, optical coherence tomography (OCT), three-dimensional skin imaging analysis, and POSAS.
- Study intervention: Administer treatment with LED-RL phototherapy device and mock therapy device on either side of the face in accordance with the randomization and treatment protocol.
- Safety monitoring: Record adverse events as reported by the subject or observed by the investigator, if applicable.
- Obtain post-treatment session photographs.

Wednesday (Visit 3, POD 7): Same procedures/evaluations as previous visit (except for obtaining baseline data for efficacy assessments), plus a review of the subject's diary of adverse events.
Friday (Visit 4, POD 9): Same procedures/evaluations as previous visit.
Monday (Visit 5, POD 12): Same procedures/evaluations as previous visit.
Wednesday (Visit 6, POD 14): Same procedures/evaluations as previous visit.
Friday (Visit 7, POD 16): Same procedures/evaluations as previous visit.
Monday (Visit 8, POD 19): Same procedures/evaluations as previous visit.
Wednesday (Visit 9, POD 21): Same procedures/evaluations as previous visit.
Friday (Visit 10, POD 23): Same procedures/evaluations as previous visit.

Follow-Up Visits after Conclusion of Treatment Period:
Follow-up visits will be scheduled on approximately POD 30 (Visit 11) and POD 90 (Visit 12). The exact timing of the follow-up visits is not strict and may occur up to ± 1 week from the proposed POD.

1 Month Follow-Up (Visit 11, approximately POD 30) and 3 Month Follow-Up (Visit 12, approximately POD 90)

- Record adverse events since last study visit, if applicable.
- Obtain standardized digital photographs of periauricular incisions/scars for visual analogue scale (VAS) scoring.
- Measure physical scar characteristics using OCT and three-dimensional skin imaging analysis.
- Measure collagen and water concentration using a spatially resolved spectroscopy probe.
- Measure scar pliability using indentation instruments to quantify skin elasticity and induration.
- Perform subjective scar evaluation using the Patient and Observer Scar Assessment Scale (POSAS). The observer assessment is done by a blinded investigator (the treating surgeon).

At the 1 month follow-up visit, optional post-treatment skin biopsies will be obtained if the subject has provided consent. Subjects may decline biopsy and remain in the study.

Final Study Visit (Visit 13, approximately POD 180): Same procedures/evaluations as previous visit, plus an exit questionnaire to elicit the subject's feedback.

The final study visit is a 6-month follow-up at approximately POD 180. However, subjects will have the option to attend a 12-month follow-up (approximately POD 365) that consists of the same procedures/evaluations as the previous visit. While a study endpoint at 12 months would be ideal for long-term monitoring of clinical changes during the scar maturation process, subject attrition is a concern and we anticipate that subjects may have difficulty returning to clinic 1 year post-surgery.

2 INTRODUCTION

2.1 STUDY RATIONALE

The goal of this study is to evaluate the safety and clinical efficacy of LED-RL as a treatment modality to reduce skin scarring after mini-facelift surgery. We hypothesize that LED-RL phototherapy has the potential to mitigate skin fibrosis by modulating key cellular and molecular mechanisms of fibrosis, including fibroblast proliferation and collagen formation.

Skin fibrosis is a common complication in the wound healing process and remains a challenge in clinical medicine. Clinical research on post-surgical scarring indicates that early intervention on new wounds (i.e., in the immediate post-operative period) is more beneficial than treatment of mature scars, as prevention of scar formation is the key to avoidance of poor scarring.[2] Despite high demand for early intervention to minimize scarring, there are few effective and durable treatments available. Skin fibrosis secondary to surgery and other trauma is due to abnormal wound healing, wherein fibroblasts remain pathologically active and continue to proliferate and produce collagen, resulting in hypertrophic scars and keloids. According to our *in vitro* data, LED-RL at fluences above 320 J/cm^2 demonstrates anti-fibrotic properties, limiting fibroblast proliferation and collagen deposition in the dermis.[3,4] Furthermore, in two Phase I clinical trials, we demonstrated the safety and tolerability of LED-RL at fluences up to 480 J/cm^2 on normal human skin (unpublished data).

We propose to conduct a randomized split-face study to test the anti-fibrotic effects of LED-RL phototherapy in subjects who have decided to undergo elective mini-facelift surgery, using the periauricular skin incisions as the treatment sites. We chose this surgical wound model given that periauricular skin is at increased risk of scarring, yet is inconspicuous and a split-face study design allows subjects to serve as their own controls.

2.2 BACKGROUND

Skin fibrosis is a significant global health problem with an estimated incidence of greater than 100 million persons affected per annum worldwide and few effective treatment options.[5] Characterized by excessive fibroblast proliferation and collagen deposition, skin fibrosis is involved in a variety of pathologic processes ranging from exuberant scar formation secondary to surgery or trauma, as in hypertrophic and keloidal scars, to immune-mediated processes such as scleroderma and chronic graft versus host disease. As highlighted by quality-of-life studies, skin fibrosis is associated with significant psychosocial stress and socioeconomic burden due to the functional, aesthetic, and emotional impacts it has on patients' lives.[5–7] Scar prevention and reduction is largely an unmet clinical need, as there is no universal consensus on optimal scar management. Currently, there are few non- or minimally

invasive and effective interventions or devices shown to limit scarring,[1,8–10] underscoring the impact and importance of therapeutic advances to achieve scarless wound healing.[11] By studying the clinical and cellular effects of red light photobiomodulation on skin fibrosis, we aim to pioneer LED-RL as a paradigm changing, cost-effective, non-invasive treatment to prevent scarring and identify the associated molecular mechanisms using high-throughput screening arrays.[12]

The effects of visible light, while common in our environment (visible spectrum accounts for 44% of total solar energy),[13] remain undefined. An important safety feature of visible red light (600 nm to 700 nm) is that it does not generate pro-carcinogenic DNA damage, unlike ultraviolet (UV) light.[13] Visible light also has other advantages over UV light, as light of a longer wavelength can penetrate deeper into skin. Red light penetrates up to 4 mm in depth, reaching the entirety of the dermis where skin fibrosis occurs.[14,15] Recently published clinical observations indicate that red light in combination with other modalities such as photosensitizers can lessen skin fibrosis.[16–18] However, our *in vitro* data suggest that red light can function as a standalone treatment, eliminating the side effects of chemical photosensitizers and the potential long-term harm of UV therapy. Furthermore, commercially available LED units exist and are FDA-cleared for various medical and aesthetic dermatologic conditions.[19] Thus, clinical translation for use in skin fibrosis could occur relatively quickly following demonstration of safety and efficacy. Developing LED-RL phototherapy as a treatment for skin fibrosis would represent an important therapeutic advance in scar modulation as it would offer many advantages over current treatment strategies. It lacks the serious systemic side effects associated with immunomodulatory agents (such as oral steroids); avoids the need for invasive, painful injections with anti-fibrotic agents (such as intralesional steroids, 5-fluorouracil and bleomycin); and eliminates the UV-induced DNA damage associated with skin cancer and photoaging that can result from UVA/UVA1 and UVB/narrowband UVB phototherapy.

There is limited knowledge on the mechanism(s) of red light photobiomodulation of skin fibrosis. Prior to our studies, *in vitro* data regarding the anti-fibrotic effects of LED-RL were lacking. Our studies are the first to indicate that LED-RL alone may modulate skin fibrosis, therefore there is a paucity of information in this emerging field of investigation. Based on our *in vitro* data, we proposed a mechanistic pathway demonstrating the cellular effects of red light photobiomodulation of skin fibrosis. Targets for modulating cutaneous fibrosis include: 1) reducing cellular fibroblast proliferation and migration, 2) inhibiting pro-fibrotic cytokines and growth factors such as transforming growth factor-beta (TGF-β), SMADs 2/3, 4, 7, connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF), 3) modulating inflammatory processes associated with fibrosis such as free radical reactive oxygen species (ROS), and 4) decreasing biosynthesis of procollagen.[20,21] Since red light irradiation can modulate some of these targets,[22–24] it is an apt therapeutic candidate for anti-fibrosis evaluation.

The hypothesized mechanistic pathway demonstrating the possible cellular effects and pathways of red light photobiomodulation of skin fibrosis is as follows: LED-RL phototherapy upregulates heme-copper photoacceptors in cytochrome c within mitochondria, leading to an upregulation of the electron transport chain function resulting in intramitochondrial changes: increased generation of ATP and ROS, increased intramitochondrial calcium, and increased mitochondrial membrane potential.[25] Increased ROS frees bound TGF-β1 from latency binding proteins, allowing TGF-β1 to bind to TGF-β receptor II, initiating a pro-fibrotic cascade that modulates downstream SMADs, growth factors CTGF, PDGF, and bFGF, modulating proliferation and collagen biosynthesis, as low levels of TGF-β1 have been shown to increase fibroblast proliferation while higher concentrations inhibit fibroblast proliferation.[26–36] Specific microRNA are associated with skin fibrosis,[37] however it is unclear if LED-RL

modulates these microRNA (currently being researched by our laboratory). Connection of these intracellular signaling pathways led to our hypothesis that LED-RL phototherapy can alter cellular mediators of fibrosis.

In addition to testing the clinical efficacy of LED-RL to prevent skin fibrosis, we aim to elucidate the molecular changes that occur *in vivo* as a result of treatment. Understanding the molecular mechanisms of red light photobiomodulation will allow for refinement and optimization of LED-RL treatment and contribute to a broader understanding of wound healing and scar formation, with potential for applicability in developing LED-RL based treatments to prevent pathologic scars affecting patients following trauma, burns, and surgery.

We previously sought to address gaps in knowledge by demonstrating LED-RL anti-fibrotic effects *in vitro* and elucidating some of the underlying mechanisms involved in LED-RL photobiomodulation of skin fibrosis. LED-RL doses above 320 J/cm^2 decrease fibroblast cell counts, DNA synthesis, and decrease procollagen synthesis and collagen, without increasing apoptosis or necrosis. We demonstrated similar findings in two keloid scar-derived fibroblast cultures.[4] These effects appeared to be mediated by alterations in cell cycle. Furthermore, these mechanistic studies demonstrated that LED-RL modulates the PI3K/AKT pathway, and LY294002, a PI3K/AKT inhibitor, blocks LED-RL inhibitory effects on cell function (migration), suggesting that LED-RL functions in-part via this pathway. Additional mechanistic studies did not demonstrate LED-RL effect on ERK, an alternate pathway involved in fibrosis.

Animal studies also demonstrate safety of LED-RL *in vivo*. We have tested LED-RL fluences up to 320 J/cm^2 in mice without any noticeable adverse events. However, given the anatomical differences in mouse skin (e.g., the epidermis and dermis measure 0.18 mm to 0.51 mm thick in mice compared to 4 mm thick in humans),[38] it is predicted that the human equivalency dose will be much higher and thus our LED-RL starting dose in Phase I studies was conservative. We proceeded directly to human clinical study based on the rationale that animal models of skin fibrosis do not recapitulate human scarring well, combined with the high level of importance of evaluating the safety and photobiomodulatory anti-fibrotic properties and mechanisms associated with LED-RL on normal human skin and human models of surgical scarring to yield the maximum patient benefit.

While commercially available LED light therapy devices exist and are FDA-cleared for the treatment of various dermatological conditions such as facial wrinkles and acne, none are approved for the treatment of scars. To our knowledge, no clinical trials have been performed to determine the safety and efficacy of LED-RL for the treatment of skin fibrosis. Therefore, we intend to study LED-RL as a sole therapeutic modality for skin fibrosis, with the goal of pioneering LED-RL as a safe, cost-effective, portable, at-home therapy to reduce cutaneous scars.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

We do not anticipate significant social, financial, legal or other risks to subjects from the proposed study, and the likelihood and seriousness of these risks occurring is low. Possible psychological risks include study boredom during longer treatment sessions and dissatisfaction or disappointment with the cosmetic outcomes of the mini-facelift surgery and/or post-treated incisions. Subjects may

experience financial loss (e.g., transportation costs) and loss of time due to study participation. Although there is minimal legal risk and all appropriate safeguards are in place to prevent dissemination of protected health information (PHI), subjects may experience breach of data.

Physical risks of the proposed mini-facelift surgery are as follows: bleeding, hematoma formation (bruising), infection, facial nerve injury with weakness, change in skin sensation, cutaneous or fat necrosis, seromas (fluid collection), scarring, poor wound healing, temporary or permanent alopecia (hair loss) at incisions, swelling, pain, skin sensitivity, skin contour irregularities, skin discoloration, facial asymmetry, irritation from sutures, wound separation, and discomfort from injection of local anesthesia. Any of the above risks may be immediate or long-term complications. As mentioned above, the subject may feel disappointed or upset by unsatisfactory results or surgical complications. The selection of mini-facelift surgery to serve as the surgical wound/scar model for this study is explained in *Section 2.3.3: Assessment of Potential Risks and Benefits*.

Risks of the local anesthesia used during the mini-facelift surgery include: allergic reaction, bleeding, discomfort from the injection, injury from the needle used to inject the anesthesia, paresthesia (abnormal skin sensation such as burning, numbness, and tingling), and toxicity from excessive dose of anesthesia given.

Physical risks of the proposed LED-RL phototherapy include potential toxicity and lack of efficacy. There is low risk of infection as we will not treat non-intact skin (since presence of open wound(s) at the treatment site is an exclusion criterion) and all LED-RL phototherapy devices will be cleaned with disinfectant before and after each treatment session. Common expected post-treatment effects include mild erythema (redness), warmth, and swelling that is transient (i.e., lasts less than 24 hours). Subjects may have tenderness or swelling at the treatment site. Since darker skin pigment absorbs more light energy in the epidermis, it is possible that subjects with darker skin types may experience a greater sensation of epidermal warmth, textural changes, or dyspigmentation. Due to the split-face study design (i.e., only one surgical incision receives LED-RL phototherapy), there is a possibility of uneven cosmetic results of the scar appearances at the conclusion of the study. That is, the scar on one side of the face may look better, worse, or the same as the scar on the other side.

Potential serious adverse events including, but not limited to, second-degree or higher skin burning, blistering, persistent swelling, persistent pain, ulceration, change in sensation, mucle weakness, or worsening of surgical scar will be monitored and the trial will be halted early if necessary. While we have tested the effects of LED-RL on fibroblasts, it is not known whether LED-RL alters the function of other cell types *in vivo*. At the doses studied, there may be inhibitory effects on other cells found in the epidermis and dermis such as keratinocytes, melanocytes, endothelial cells, neurons and immune cells. Due to the location of LED-RL phototherapy on the face, there is a theoretical risk of neurological side effects such as headaches, malaise, fatigue, and change in hearing.

We do not anticipate any physical risks of the mock irradiation since it does not generate light and there were no adverse events associated with the mock treatment device in the Phase I studies. However, due to the split-face study design, subjects may have uneven cosmetic results between the two periauricular incision sites. The scar on one side of the face may appear better, worse, or the same as the scar on the contralateral side, depending on the effects of the study intervention.

A proposed component of this study is optional skin biopsies for histological examination. Though a skin biopsy is a generally safe procedure, potential risks include bleeding, bruising, scarring, pain, skin sensitivity, and infection.

2.3.2 KNOWN POTENTIAL BENEFITS

Potential benefits of mini-facelift surgery include improvement in visible signs of aging on the face (e.g., improvement in wrinkles and laxity) and improved self-esteem from a good cosmetic outcome.

Known potential benefits of the Omnilux new-U for LED-RL include reduction of periorbital wrinkles, the current FDA indication for use. The potential benefit of scar prevention is being studied in this protocol.

Patients with post-surgical scars and skin fibrosis in general have limited treatment options. Even with treatment, skin fibrosis often persists and recurrence rates are high. If LED-RL phototherapy is effective at preventing hypertrophic or keloidal periauricular scarring, subjects will benefit from improved scar appearance and symptomology. This benefit by patients will in turn aid medicine and science.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Although no surgical procedure is without complications, facelift surgery is considered to be a safe procedure with a low incidence of major complications, with hematoma (1.1%) and infection (0.3%) being the most common but still occur infrequently.[39] We chose mini-facelift incisions as the surgical wound model for the following reasons: it is considered to be a safe procedure, performed in-office with tumescent local anesthesia; it offers an aesthetic benefit to patients who are interested in facial rejuvenation; and the surgical incisions are made in relatively inconspicuous areas near the earlobes and are symmetrical, allowing for a split-face study. We believe that given the low incidence of complications of this commonly performed elective cosmetic procedure, combined with the fact that the procedure in this protocol is a minimal incision facelift (as opposed to the traditional full facelift that requires more extensive incisions and longer recovery time), the value of information to be gained by studying this surgical wound model outweighs the risks involved.

LED-RL phototherapy is also considered to be a safe procedure that is done routinely in outpatient clinics and even at home. We will monitor for any unforeseen adverse events caused by LED-RL and follow study stopping rules, as described below. LED-RL phototherapy is completely non-invasive, does not cause thermal or chemical damage, and does not have post-procedure down time. The risk of side effects is minimal and those that do occur are usually transient and minor (e.g., mild erythema lasting less than 24 hours). We believe that the value of the information to be gained in this study outweighs the potential risks involved in LED-RL phototherapy, given that the treatment device is FDA-cleared for over-the-counter use on the face and has been clinically proven to be safe and well tolerated at the proposed treatment doses.

The skin biopsies are optional and will be used for research purposes only. Minimal risk of scar will be incurred by subjects undergoing biopsy, as a 2 mm punch biopsy tool (the smallest sized instrument that can be used to obtain useful histological and molecular analysis) was selected to minimize

discomfort and reduce the risk of scarring. The 2 mm size is so small that the punch biopsy defect (skin wound) does not require suturing and heals by secondary intention.

During the study, subjects are asked to refrain from using scar treatments to both periauricular incision sites, as to not confound the results of the study. They will be counseled on standard postoperative care of the incisions to optimize wound healing. Subjects are allowed to seek scar treatment, if desired, at the conclusion of the study (i.e., after the final study visit at 6 months post-surgery). Facial skin is known to heal well, and thus have lower risk of abnormal scarring, compared to other body sites due to its rich vasculature and low tensile forces.[40,41] Since most cutaneous injuries (such as surgical incisions) heal with a normotrophic scar over time,[41,42] the study's requirement that subjects wait 6 months post-surgery to seek scar treatment is not expected to cause irreversible health problems or extreme suffering. A normotrophic scar is defined as a flesh-colored, flat scar (as opposed to hypertrophic scars or keloids, pathological scars that are elevated and can be symptomatic).[42,43] Furthermore, the remodeling phase of skin wound healing can take over a year, meaning that the scar maturation process is lengthy and the final scar appearance may not be known until 6 months to 2 years after surgery.[44,45] It is important to note that should pathological scarring develop in a subject, it will not be evident until several months post-surgery, as this is the general timepoint at which a hyperplastic response (i.e., abnormal scar tissue formation) is observed.[41] In clinical practice, it is recommended that hypertrophic scars be allowed the opportunity to regress on their own before initiating aggressive anti-scarring treaments such as scar revision surgery.[43,46]

Stopping Rules: Subjects will have the ability to leave the study at any time without penalty for discontinuation of participation. Adverse events, serious adverse events, and unanticipated problems (as described in *Section 8: Study Assessments and Procedures*) will be monitored and the trial will be halted early if necessary to protect subjects. Any life threatening events and/or deaths (grade 4 and 5 toxicity) attributable to the study protocol will result in halting the study. Toxicity data and adverse events will be monitored by the PI and the IRB.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To evaluate the clinical efficacy of LED-RL phototherapy in the prevention of skin fibrosis in subjects undergoing elective mini-facelift surgery	**The difference in quantitative scar pliability between the LED-RL treated and control incision sites** Skin elasticity (stiffness) and induration, which reflect scar pliability, are objectively quantified with two different indentation instruments, the ElastiMeter and the SkinFibroMeter. These non-invasive handheld tools measure vertical deformation on the skin surface; the indenter is pressed quickly onto the skin and the sensors measure the force resisting the strain. Scar tissue is less supple than normal skin due to scar thickness and inferior quality of collagen	Various objective scar assessment tools are available to assess biomechanical properties of the skin, including pliability, which correlates with degree of skin fibrosis.[47–52] These devices have shown less pliability in scars as compared to normal controls and are commonly used to measure the efficacy of scar treatments.[53] The ElastiMeter demonstrates accuracy and reliability to assess skin elasticity.[54] The SkinFibroMeter has good reliability compared with other elasticity measurement devices and has been applied in clinical studies to assess skin fibrosis.[55,56]

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	architecture. Elasticity and induration measurements are performed prior to treatment and at follow-up visits on POD 30, 90, and 180.	
Secondary		
To evaluate the histological, ultrastructural, and molecular changes that occur *in vivo* as a result of LED-RL phototherapy	**Histological and molecular analyses of treated and control skin specimens** Skin samples from LED-treated and mock-treated incision sites are obtained for histology and molecular studies. Tissue specimens from POD 30 (post-treatment) are compared to those from POD 0 (pre-treatment). Histological examination includes quantification of Ki-67 positive fibroblasts and qualitative assessment of collagen. Molecular testing includes RNA-Seq, microRNA arrays, and quantitative real-time PCR (qRT-PCR).	Non-invasive tests to evaluate cellular features of skin fibrosis are limited. Our *in vitro* data showed that high fluence LED-RL decreases fibroblast cell counts in keloid scar-derived fibroblast cultures[4] and that LED-RL modulates the PI3K/AKT pathway.[57] To determine the mechanism associated with LED-RL anti-fibrotic effects *in vivo*, biopsies of the dermis (where skin fibrosis occurs) are needed to conduct histological examination and molecular studies.
To assess for clinically meaningful changes in scar characteristics after LED-RL phototherapy	**Difference in the Patient and Observer Scar Assessment Scale (POSAS) scores between the treated and control incision sites** Subjective scar evaluation is performed using the POSAS, a standardized and validated tool to assess scar quality.[58–60] The observer scar assessments are completed in-clinic by a blinded investigator in conjunction with subject assessments at multiple time points (follow-up visits on POD 30, 90, and 180). Each parameter on the POSAS is scored from 1 to 10, where 1 is "normal skin" and 10 is the "worst imaginable scar".	The POSAS is the most frequently used scar assessment scale for post-surgical scars[61] and is often used as a primary outcome measure for treatment efficacy in clinical trials. The POSAS was devised to quantify scar appearance in response to treatment and demonstrates internal consistency, inter-observer reliability, and agreement.[53,58] Importantly, the POSAS incorporates the patient's judgment of scar appearance and includes subjective symptoms of pain and pruritus, allowing for a more comprehensive evaluation in comparison to an evaluation by clinicians alone.
	Difference in the photograph-based Visual Analogue Scale (VAS) scores between the treated and control incision sites An independent panel of two blinded dermatologists will evaluate standardized split-face scar photographs, using a validated VAS scoring and ranking system.[53,62,63] Each scar dimension is rated on a 10 cm VAS such that	The VAS has demonstrated high observer reliability and internal consistency in expert panel evaluations.[53,63] The VAS has been used in various scar trials[53] and Duncan et al. showed that the VAS scar scoring and scar ranking methods are consistent, reliable, valid, and feasible for assessing linear scars.[62]

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	higher numerical scores correlated with more severe scars.	
	Difference in objective measurements of key scar characteristics between the treated and control incision sites A diverse set of novel, non-invasive skin analysis tools will be used to objectively measure key scar characteristics: collagen and water concentration, wrinkles, texture, diameter, area, volume of elevation, pores, pigmentation, and vascularity. The VivoSight uses multi-beam OCT technology to provide high-resolution cross-sectional images and measure scar depth, collagen density, and surface roughness.[64] The Dermo is a spectroscopy probe that measures collagen and water concentration in the dermis. The Antera 3D camera and Cherry3 camera use multi-directional illumination to reconstruct three-dimensional images of the skin surface for instant topographic and colorimetric analyses. These objective efficacy assessments will be done prior to treatment initiation and at follow-up visits.	OCT has been used to non-invasively evaluate skin fibrosis at high resolution without discernible effect on tissue.[65] This imaging technique is highly suited to fibrosis assessment and may represent a diagnostic alternative to punch biopsies.[66] Three-dimensional skin imaging analysis provides quantitative measurements of various scar tissue indicators such as pigmentation, vascularity, texture, and volume of depressions and elevations. Because it is an objective analysis tool, it provides quantitative evidence of therapeutic results, avoiding the influence of subjective evaluation that most scar assessments are based on.[67–69] It has been used to assess therapeutic efficacy of various interventions on keloids and burn scars.
To continue to assess the safety of LED-RL phototherapy on human skin	The safety profile is determined by the incidence of common expected post-treatment effects, adverse events, serious adverse events, and unanticipated problems throughout the study. Special attention will be paid to the identification of any differential safety findings based upon skin type (i.e., race and ethnicity), given the findings of our previous Phase I trials.	While two phase I studies demonstrated the safety of LED-RL in healthy subjects, the safety of LED-RL in a mini-facelift surgical wound model may differ from the safety on normal human skin. In addition, differential safety effects of LED-RL on different skin types have not been described previously, but our previous research suggest that darker skin types may be more photosensitive to LED-RL.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a Phase II clinical trial, following two previous Phase I studies of LED-RL on human skin in healthy subjects (STARS 1 and STARS 2). We will conduct a dose-ranging, randomized, parallel group, split-face, single-blind, mock-controlled study of LED-RL in eligible subjects who have decided to

undergo elective minimal incision facelift (i.e., mini-facelift) surgery. We hypothesize that LED-RL phototherapy may be a safe, well tolerated, and effective treatment for skin fibrosis.

The trial will be conducted at a single site: the Laser, Aesthetics and Body (LAB) Institute at SUNY Downstate Medical Center. The LAB Institute is part of the Center for Photomedicine, Department of Dermatology, and is located at University Hospital of Brooklyn. The mini-facelift surgery is a simple excision of redundant skin (i.e., excess lax facial skin) with wound closure. The surgery is done in-office in a procedure room within the LAB Institute, which is equipped with LED surgical lighting and stocked with standard care supplies, medications, and surgical instruments needed for dermatologic surgical procedures done in an ambulatory setting. The surgery is performed under tumescent local anesthesia by a board-certified dermatologic surgeon (Dr. Daniel Siegel) at the baseline visit, referred to as post-operative day (POD) 0. Following the mini-facelift surgery, subjects will be counseled on standard postoperative wound care and be provided with a handout of instructions. Subjects are asked to adhere to the postoperative care instructions to optimize skin wound healing and to refrain from using scar treatments at the incision sites during the study.

Subjects will use commercially available, FDA-cleared LED-RL phototherapy treatment devices (Omnilux new-U; GlobalMed Technologies, Glen Ellen, CA) aimed at the periauricular skin (i.e., the site of incisions from mini-facelift surgery). The Omnilux new-U will be held in place using a provided hair net that has been used by other researchers to stabilize facial positioning of LED-RL phototherapy units. The maximum recommended starting dose (MRSD) of 160 J/cm^2 is based on previously published maximum doses of LED-RL that demonstrated safety with no adverse events in clinical studies.[70,71] The highest dose to be tested is 480 J/cm^2, which was found to be the maximum tolerated dose (MTD) in our Phase I studies (unpublished data). The MTD is defined as the dose level below the dose producing unacceptable but reversible toxicity and is considered to be the upper limit of subject tolerance.

A total of 30 subjects will be randomly allocated into three treatment groups of 10 subjects to receive LED-RL phototherapy at the following doses: 160 J/cm^2, 320 J/cm^2, or 480 J/cm^2. Based on safety data obtained in our Phase I studies, we concluded that skin of color individuals (as determined by race and ethnicity) may be more photosensitive to LED-RL compared to Caucasian non-Hispanic individuals. This novel description of possible differential cellular effects of red light on skin is based on findings that LED-RL doses up to 320 J/cm^2 was safe in all skin types, but one African-American subject had an adverse event of a blister with LED-RL at 480 J/cm^2. Because no definitive conclusions may be drawn due to the limited sample size (1 subject who developed a blister out of 3 subjects who received the same treatment in that group), and the dose-escalation study design precluded continued administration of LED-RL at 480 J/cm^2 (i.e., no further subjects were enrolled in that treatment group due to the adverse event), this trial will test the MTD of 480 J/cm^2 in all subjects regardless of skin type.

In this study, skin type is determined by the National Institute of Health's definitions for racial and ethnic categories.[72] For the purposes of monitoring safety in relation to skin type, any individual who self-identifies with a race/ethnicity (or any combination thereof) other than "Caucasian, not Hispanic or Latino" will be considered to have skin of color.

The treatment side (right face versus left face) will also be randomized to control for effects of uneven sun exposure. The untreated side will receive mock therapy with sham irradiation via a mock device that looks, sounds, and feels similar to the treatment device, but does not produce light. Subjects will

be blinded to the LED-RL device versus mock device as the treatment site is outside of their range of view and they will wear safety goggles.

Beginning scar reduction therapy at 1 week after surgery (defined as POD 4 to POD 8) is a validated intervention time point for limiting surgical scars.[1] Subjects will receive their treatment sessions in-office three times weekly, a standard phototherapy regimen,[73–75] starting at 1 week post-surgery for a total of nine treatment sessions. This treatment schedule (frequency and duration) is identical to the schedules used in our Phase I studies. Each treatment session will be observed by an investigator to monitor for any adverse events and to ensure safety and adherence.

During the study, subjects will be asked to avoid scar treatments to both periauricular incision sites. Scar treatment options include, but are not limited to, silicone gels/sheets, intralesional corticosteroids, 5-fluorouracil, laser therapy, radiotherapy, cryotherapy, bleomycin, mitomycin C, imiquimod, pressure therapy, adhesive microporous hypoallergenic paper tape, onion extract, massage therapy, over-the-counter topical emollients for scars, laser therapy, and surgical revision.[76] As described above, subjects will be counseled on proper wound care during the postoperative period to facilitate appropriate wound healing. Refer to *Section 10.2: Additional Considerations* for a discussion of available scar treatments at the conclusion of the study.

Following the study intervention period, subjects will have follow-up assessments on approximately POD 30, 90, and 180. At each follow-up visit, scar characteristics will be recorded objectively and subjectively through skin elasticity and induration measurements, tissue histology, photographic evaluation, skin imaging analysis, collagen and water concentration measurements, OCT, and standardized patient and observer assessments as described in *Section 3: Objectives and Endpoints* and *Section 8: Study Assessments and Procedures*.

Skin specimens will be obtained via elective 2 mm punch biopsy on POD 0 (prior to surgery) and POD 30 (first follow-up visit after completion of LED-RL treatment). Skin specimens will be used for histological examination and molecular studies via RNA sequencing, microRNA analysis, and qRT-PCR.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

The methodology of a dose-ranging, randomized, parallel group, split-face, single-blind, mock-controlled study offers several advantages.

A dose-ranging study design is implemented as the safety of LED-RL phototherapy in a surgical wound model may differ from the safety in normal skin. For example, higher fluences determined to be safe in normal skin may potentially cause problems in post-surgical/scarred skin, such as dehiscence. Thus, the MTD established in our Phase I studies serves as the upper limit of treatment dose in this study.

The split-face study design allows each subject to serve as his or her own control, allowing for a within-subject comparison of clinical efficacy between the treated side and mock-treated side. That is, any observed improvements in scar characteristics can be attributed to the treatment. The split-face design also eliminates the need to consider inter-individual differences in wound healing as a confounding factor when comparing different treatment arms. It is important to note that in the prospective evaluation of scar reduction therapy, it is assumed that if left untreated, the wounds on the treated side and untreated side would heal with identical scars. Since wound healing and scar

formation are influenced by many variables, the selection of a self-controlled study design is favored to allow detection of true treatment effects.

There are no known problems associated with the control (sham irradiation), since the mock device is designed to simulate the treatment device without actually producing light.

The mini-facelift with periauricular incisions is an ideal model for studying wound healing and skin fibrosis because: patients are motivated to have the best wound healing outcomes and aesthetic results (i.e., minimal or imperceptible scarring), hence will likely be compliant with therapy and have a low attrition rate; patients are usually healthy in order to be suitable candidates for the surgery; and the periauricular skin is at risk of pathological scar development, yet has low skin tension and robust blood circulation to allow for optimal wound healing.

4.3 JUSTIFICATION FOR DOSE

As discussed in *Section 4.1: Overall Design*, the MRSD of 160 J/cm^2 is based on maximum doses of LED-RL that demonstrated safety with no adverse events in clinical studies.[49,50] The planned maximum dose is 480 J/cm^2, which was found to be the MTD for Caucasian non-Hispanic individuals in our Phase I studies.

Based on safety data generated by our Phase I studies, we had established a MTD of 320 J/cm^2 in individuals with darker skin color based on an adverse event of a skin blister in an African-American subject following administration of LED-RL at 480 J/cm^2. Because the occurrence of a skin blister was pre-defined as an unacceptable toxicity, further treatment sessions for subjects enrolled in the LED-RL 480 J/cm^2 treatment group were halted. That subject was the only one out of 3 subjects of different skin types (1 African American, 2 Caucasian non-Hispanic) in that particular treatment group who had experienced a blister. Therefore, while no definitive conclusions may be drawn about differential safety of LED-RL based on race/ethnicity due to the limited sample size, we hypothesized that skin of color individuals may be more photosensitive to LED-RL compared to Caucasian non-Hispanic individuals. This observation of possible differential safety of visible red light on different skin types had not been described previously. To further evaluate safety of LED-RL on skin and to explore this hypothesis, this Phase II trial is designed to allow subjects of any skin type to be randomized to the highest intensity treatment dose of 480 J/cm^2. In a joint discussion with research colleagues and the IRB, the study team decided that the adverse event of a blister, which is more likely to occur at higher fluences, is an acceptable risk since treatment-related cutaneous blistering is expected to be mild and resolve without permanent sequelae. During the informed consent process, subjects will be counseled on the potential risk of blistering and be informed that darker skin individuals may be more prone to this risk.

Please refer to *Section 2.3.3: Assessment and Potential Risks and Benefits* and *Section 7.1: Discontinuation of Study Intervention* for a discussion and description of stopping rules.

The thrice weekly treatment regimen is a standard phototherapy protocol,[52-54] which is based on guidelines for UVB phototherapy for psoriasis and is applicable to this study of LED-RL phototherapy.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit shown in *Section 1.3, Schedule of Activities (SOA).*

The end of the study is defined as completion of the last visit or procedure shown in the SOA in the trial globally. The end of the study is the point at which all required data has been collected to answer the research questions in the protocol.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

Individuals of any sex, ethnicity, and age may potentially be eligible to participate in this study. In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Desire to undergo elective mini-facelift surgery and is a suitable surgical candidate, as determined by the surgeon's clinical assessment
4. Pass a screening photosensitivity test

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Concomitant use of any photosensitizing medications (e.g., lithium, melatonin, phenothiazine antipsychotics, tetracycline antibiotics, quinolone derivative antibiotics)†
2. Light-sensitive conditions
3. Diabetes mellitus
4. Systemic lupus erythematosus
5. Current tobacco use
6. History of bleeding or coagulation disorder
7. Lax skin associated with genetic disorders (e.g., Ehlers-Danlos, cutis laxa)
8. Open wounds on the face or neck
9. Fibrotic skin disease, pre-existing scar(s), or other skin conditions affecting the periauricular skin
10. History of surgery or procedure involving or affecting the periauricular facial skin within the past 6 months (e.g., facial plastic surgery, dermal fillers, physician-strength chemical peels, neuromodulator treatment, laser skin resurfacing), or plan to have such procedures during the study period
11. Tattoos that cover the proposed treatment sites on the periauricular skin
12. Any other medical condition(s) that could be compromised by exposure to the proposed treatment

† Refer to *Section 6.5: Concomitant Therapy* for the full list of prohibited medications to be reviewed on case report forms.

5.3 LIFESTYLE CONSIDERATIONS

During this study, participants are asked to:

- Abstain from smoking
- Refrain from use of any scar treatments (e.g., silicone gel/sheets, intralesional corticosteroids, massage therapy, topical emollients, laser therapy, surgical revision) on the periauricular incision sites
- Avoid any elective cosmetic procedure that would affect the facial skin

If a prohibited medications, treatments, or procedures are indicated for standard of care, the participant will be withdrawn early from the study. Exceptions include, but are not limited to, use of a photosensitizing medication after the conclusion of the intervention period (i.e., after all LED-RL phototherapy sessions are completed).

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of use of prohibited medications that have subsequently been discontinued may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Potential subjects who are interested in elective mini-facelift surgery will be recruited through IRB-approved flyers on bulletin boards, physician referrals, websites (SUNY Downstate Department of Dermatology webpage, SUNY Downstate Clinical and Translational Science Center “Clinical Trials List”, StudyPages.com, and ClinicalTrials.gov), social media, website ads, radio ads, and/or newspaper ads. Potential participants will be identified by their expressed interest in a mini-facelift or perceived suitability for the surgery (i.e., they have excess lax facial skin and desire a mini-facelift). Aside from physician referrals and direct recruitment from clinic, recruitment materials will be distributed or made available to the general public. That is, potential participants may learn about the study and approach or contact the research team directly. We anticipate screening up to 150 potential participants and ultimately enrolling 30 subjects.

At the initial screening visit, investigators will explain the study, provide relevant literature and information, and answer all questions. Written informed consent will be obtained prior to any study-related procedures or interventions. We will indicate that the subjects will receive the same standard of clinical care options regardless of whether they decide to participate in the study. Subjects will be informed that the costs of the surgery are covered by the research study and that participation is voluntary, allowing them to withdraw from the study at any time.

Measures to minimize undue influence: Due to the nature of this research study, specifically the provision of an elective cosmetic procedure at no cost to the subject, measures are in place to minimize undue influence. The fact that the cost of the surgery is covered by the Department of Dermatology will not be advertised in recruitment materials. That is, we are not emphasizing "free surgery" as a way to gain the attention of potential subjects. In the recruitment materials, we call for volunteers who are considering or interested in a mini-facelift, without commenting on whether the surgery itself is part of the study. The fact that the surgery will be provided at no cost for enrolled subjects is disclosed during the informed consent process (i.e., after a potential subject makes contact with the research team to express interest in the study and comes in-person for the screening visit). With this recruitment strategy, we will be able to screen potential subjects who are already aesthetically motivated and inclined to undergo cosmetic surgery such as a mini-facelift, regardless of the opportunity provided by this study. During the informed consent process, the individual's comprehension of risks, benefits, and outcome expectations are of paramount concern when determining appropriate candidates for the mini-facelift. In this study, there is an ethical concern that an offer of no-cost surgery may impair an individual's judgment such that they do not fully consider risks and thus engage in activities that contravene their interests. To minimize this risk, we will conduct an assessment of the individual's understanding and appreciation of the surgery's risks/benefits and alternative options to ensure that they demonstrate decision-making capacity. We will emphasize that research participation is voluntary, that the subject can withdraw at any time, and that the decision whether or not to participate will have no impact on the availability of care. Known benefits of the mini-facelift and the study intervention will be stated accurately but not exaggerated; potential or uncertain benefits will also be stated as such, with clear language indicating how much is known about the uncertainty or likelihood of these potential benefits. For example, we will ensure that the potential subject has reasonable expectations about the cosmetic outcome of the mini-facelift; they should understand what the mini-facelift can and cannot do to change physical appearance. We will seek consent only under circumstances that provide the prospective subject sufficient opportunity to consider participation and that minimize the possibility of undue influence.

To minimize potential for drop-out due to scheduling conflicts, subjects will be directly asked prior to enrollment if they are willing and able to be compliant with therapy (i.e., be able to attend all proposed study visits on the schedule). We will educate subjects regarding the benefits and risks of the study, as well as importance of attending all treatment sessions and follow-up visits for subject safety. We will guard against drop out by confirming study visits with subjects in advance to ensure no scheduling conflicts via multiple methods of communication including phone calls, email, and text message reminders. To minimize subject burden and enhance retention, we will make every attempt to accommodate subject preferences in scheduling time (e.g., treatment sessions in the mornings only). All treatment sessions and follow-up visits will be in the same location.

We hypothesize that drop-out will be low, as we hope that subjects are interested in and motivated to limit post-surgical scarring for functional and aesthetic reasons.

Subjects will be compensated for study participation. A total amount of $375 will be given in the form of gift cards and/or checks. Subjects will receive $100 at the end of each week during the 3-week treatment period, plus $25 for each of the 3 follow-up visits. In the event that a subject withdraws early from the study, the payment will be prorated based on the number of study visits completed prior to withdrawal.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

The LED light source is the Omnilux new-U handheld LED phototherapy device, which is commercially available and FDA-cleared for the treatment of periorbital wrinkles. This device has not been approved or cleared for the indications the protocol is designed to investigate (skin fibrosis). The device has a 3-position switch that allows the user to select the red light output (switch down).

The mock therapy device emits no irradiation. The mock therapy device has a ventilation fan and functional resistors designed to sound, look, and feel (i.e., temperature matched) similar to the phototherapy treatment device, but does not emit LED-RL.

6.1.2 DOSING AND ADMINISTRATION

Randomization:
A computer-based randomization algorithm will be used to assign subjects to three different treatment groups to receive LED-RL at the following doses: 160 J/cm^2, 320 J/cm^2, or 480 J/cm^2. The treatment side (right face versus left face) will also be randomized using the same algorithm. The same dose will be maintained throughout all treatment sessions for each individual subject, with no dose escalation. Refer to *Section 6.3: Measures to Minimize Bias* for a detailed description of the randomization protocol.

Treatment Protocol:
The subject will be inside a private clinic examination room. The subject's periauricular skin on both sides of the face will be cleaned with alcohol pads. A surgical marking pen will be used to outline the treatment area at the start and completion of every treatment. The Omnilux new-U device and mock therapy device will be aimed at the periauricular skin and positioned in close contact with the skin (maximum of 1 cm from the skin surface), held in place by a hair net throughout the duration of the treatment. LED-RL phototherapy and mock therapy are administered simultaneously. Subjects will be allowed to receive the treatment sitting up or laying down, per personal preference for a comfortable position.

Duration of the treatment administration for each group is as follows:

- Group 1: LED-RL 160 J/cm^2 and mock therapy – 30 minutes
- Group 2: LED-RL 320 J/cm^2 and mock therapy – 60 minutes
- Group 3: LED-RL 480 J/cm^2 and mock therapy – 90 minutes

Dr. Julie Nguyen (research coordinator) will perform the phototherapy, observe the treatment session, and assess for any safety issues that may arise. Any adverse events reported by the subject or observed by the investigator will be recorded. Common expected post-treatment effects include mild erythema, warmth, and swelling, which are expected to resolve within 24 hours.

Subjects will receive LED-RL phototherapy and mock therapy sessions in-office three times weekly, starting at approximately 1 week post-surgery, for a total of nine treatment sessions. The duration of treatment administration is controlled by the investigator (i.e., the investigator will take responsibility for timing the treatment and determining when the devices are turned off to end treatment).

The Omnilux new-U has been tested to international standards to ensure that its outputs are safe for the eyes. However, subjects will wear safety goggles during the treatment sessions, as recommended by the manufacturer for patient comfort.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

The study intervention and control products were originally purchased from Photo Therapeutics. There is no distribution plan as the treatment and mock devices are already in the possession of the research team (previously purchased for the Phase I studies). Disposal of unused product is not applicable to this protocol.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

The LED light source is the Omnilux new-U (GlobalMed Technologies, Glen Ellen, CA) handheld LED phototherapy device, which is commercially available and FDA cleared for treatment of periorbital wrinkles. The device contains an array of LEDs with a transparent lens cover, a ventilation fan, a three-position switch, and a connection socket. The LED has a 4.7 cm x 6.1 cm rectangular aperture and emits visible red light (633 nm $\pm$ 6 nm) at a power density of 360.2 W/m^2 at room temperature.

The mock therapy device has a ventilation fan and functional resistors designed to sound, look, and feel similar to the phototherapy treatment device, but emits no irradiation.

6.2.3 PRODUCT STORAGE AND STABILITY

The phototherapy treatment and mock therapy devices will be stored at room temperature in a secured private clinic examination room, avoiding extreme temperatures and direct sunlight.

6.2.4 PREPARATION

The treatment and mock devices are powered by wall power supply plugged into an AC electrical outlet. The operator (i.e., investigators) will flip the selector switch to turn on the red LEDs and place the transparent lens in close contact with the treatment area, at a maximum of 1 cm from the skin surface.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Treatment Dose Randomization

A block randomization scheme will be used for balanced allocation of subjects to study arms, such that 10 subjects are assigned to each treatment group. All subjects will be randomized as they are enrolled

(i.e., sequentially in blocks of three). Study enrollment concludes when a total of 30 subjects have been randomized.

For the first subject who enrolls, Arabic numeral "1", "2", or "3" will be generated at random using a computer-based randomization tool (*www.randomizer.org*). This subject has a 1 in 3 chance of being assigned to any of the three treatment groups: "1" for Group 1 (LED-RL 160 J/cm^2), "2" for Group 2 (LED-RL 320 J/cm^2), or "3" for Group 3 (LED-RL 480 J/cm^2). The next subject who enrolls is then similarly randomized to one of the remaining two treatment groups, and the following subject who enrolls is automatically assigned to the last treatment group. This block randomization process continues until each treatment group is filled with 10 subjects.

Split-Face Randomization

Within each treatment group, the treatment side (right face versus left face) will be determined using a computer-generated randomization tool (*www.randomizer.org*). The assignment to a treatment dose and a treatment side of the face occurs simultaneously. Within each treatment group, a single set of Arabic numerals "0" to "9" will be generated, resulting in a random sequence of 10 numerals for sampling without replacement. As subjects are allocated to a treatment group, they are assigned to a numeral in this set based upon their order of enrollment. In other words, the first subject to enroll in a treatment group is assigned to the first random numeral, the second subject to the second random numeral, etc. Subjects assigned to even numerals will receive LED-RL phototherapy on the right side of the face, making the left face the control. Subjects assigned to odd numerals will receive LED-RL phototherapy on the left side of the face, making the right face the control. Within each treatment group (n=10), 5 subjects will receive treatment on the right face and the other 5 subjects will receive treatment on the left face.

Blinding

This is a single-blind study wherein the subjects are blinded to the treatment side. From the subject's perspective, the LED-RL phototherapy device and the mock therapy device are indistinguishable since 1) the treatment area is outside of their range of view and 2) the mock therapy device was designed to simulate the actual treatment device in terms of appearance, sound, and feel.

Clinicians involved in evaluating certain outcome measures (e.g., histology, POSAS, evaluation of digital photography) will be also be blinded to the treatment side. This includes the following clinicians.

- The investigator who will perform the POSAS, a subjective scar assessment scale that is scored based on clinical judgment – Dr. Daniel Siegel
- The expert panel of two independent dermatologists charged with photographic evaluation of the scars – Dr. Edward Heilman and Dr. Jeannette Jakus
- The dermatopathologist examining the skin specimen slides – Dr. Edward Heilman

The PI and research coordinator will be aware of the trial randomization codes. Unblinding may occur at the discretion of the PI. For example, the occurrence of a SAE may necessitate knowledge of the treatment side so that the subject may receive appropriate medical care. In the event of inadvertent unblinding of blinded investigators or collaborators, the data point for the outcome measure affected by the unblinding will be considered invalid. For example, if the investigator performing the POSAS inadvertently gains knowledge of the treated side, that POSAS score will be invalid and excluded from

statistical analyses due to potential bias. Intentional and unintentional breaking of the blind should be reported to the PI.

6.4 STUDY INTERVENTION COMPLIANCE

Subjects will be closely monitored during each treatment session to ensure appropriate dosing of the phototherapy device (as determined by length of treatment time) and subject's adherence to the protocol (i.e., the subject remains with both treatment and mock-treatment devices in the correct position). The exact start time and end time of each treatment session will be recorded in the case report forms.

6.5 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications, and supplements.

A review of concomitant medications will be performed at each study visit during the treatment period. Subjects will be asked to state all concomitant medications as well as to deny use of all of the prohibited medications.

Treatment with the following medications that are known to have photosensitizing properties will not be permitted, as they are relatively or absolutely contraindicated with concomitant phototherapy (adapted from Appendix 1 in the Omnilux Treatment Protocols manual), unless discussed with and approved by the investigators:

- Amiodarone
- Azathioprine
- Chlorpromazine
- Gold
- Griseofulvin
- Isotretinoin
- Lithium
- Melatonin
- Methotrexate
- Phenothiazine antipsychotics
- Tetracycline antibiotics (e.g., demecocycline, doxycycline, lymecycline, minocycline, oxytetracycline)
- Quinolone derivative antibiotics

6.5.1 RESCUE MEDICINE

N/A

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from LED-RL phototherapy does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

The data to be collected at the time of study intervention discontinuation will include the following:

- Details of any adverse events (e.g., nature, severity, relationship to study intervention, and expectedness) will be documented in the CRFs
- Digital photography of the affected site(s), if applicable

Stopping Rules: Adverse events, serious adverse events, and unanticipated problems (as described in *Section 8: Study Assessments and Procedures*) will be monitored and the trial will be halted early if necessary to protect subjects. Any life threatening events and/or deaths (grade 4 and 5 toxicity) attributable to the study protocol will result in halting the study.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request, without penalty. For example, the participant may withdraw after receiving the mini-facelift surgery but before starting treatment without being penalized for the procedure-related costs of the mini-facelift surgery.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance
- Any clinical adverse event (e.g., second-degree or higher skin burning, blistering, swelling, pain, ulceration, change in sensation, muscle weakness, worsening of surgical scar) or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Unforeseen serious adverse event (e.g., neurological symptoms lasting more than 24 hours) occurs

The reason for participant discontinuation or withdrawal from the study will be recorded on the CRF. Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will be replaced only if the enrollment period is still open.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to report to at least one scheduled study visit and is unable to be contacted by the study site staff to complete all protocol-required study procedures.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within a reasonable time window as determined by the PI (taking into consideration the time-sensitive nature of the treatment regimen and the relative flexibility of timing of the long-term follow-up visits) and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

Refer to *Section 1.3: Schedule of Activities*, for the specific timing of procedures and evaluations to be done at each study visit. All procedures and evaluations will be performed by qualified personnel.

<u>Study Specific Procedures and Evaluations for Efficacy</u>

- Digital photographs of the treatment sites will be taken immediately before and after each treatment session, as well as at each follow-up visit, to track the appearance of the incisions/scars for outcome measures. During the analysis phase, the standardized photographs will be rated in a blinded fashion by an independent panel of expert dermatologists using a visual analogue scale (VAS).
    - The VAS is a validated photograph-based scale that is presented as a 10 cm horizontal line, with the extreme ends of 0 indicating normal skin and 10 corresponding to the worst possible scar. Duncan et al. demonstrated that a VAS with scar ranking is a consistent, reliable, and valid method for linear scar assessment.[62] Micomonaco et al. further developed a VAS-based instrument that incorporates the dimensions of pigmentation, vascularity, observer comfort, contour, and overall severity for the assessment of area scars.[63]
- Objective scar characteristics will be recorded at follow-up visits using the following tools:
    - ElastiMeter and SkinFibroMeter (Delfin Technologies, Kuopio, Findland) are two non-invasive instruments used to measure skin elasticity and induration, respectively, using the same indentation principle. They each have an indenter, reference plate,

and built-in force sensors. The probe is pressed against the skin surface while the indenter imposes a constant deformation, instantly measuring the skin's elasticity.
    - VivoSight (Michelson Diagnostics, Maidstone, UK) is a high-resolution imaging technology based on multi-beam OCT that produces two-dimensional, cross-sectional, real-time imaging of the skin and provides objective measurements of tissue microstructure, including scar depth and collagen density. The scanning procedure is quick and non-invasive.
    - Dermo (Connected Physics, Orsay, France) is a novel spectroscopy probe that instantly measures both collagen and water concentration in the dermis.
    - Antera 3D (Miravex, Dublin, Ireland) is a novel skin imaging analysis device that uses reflectance mapping of different wavelengths to acquire three-dimensional images of the skin surface and objectively measures multiple skin dimensions. It accurately measures wrinkles, texture, scars, skin color, redness, and pigmentation. The software also compares images to enable progress tracking and assess therapeutic efficacy. [67,68]
        - Alternatively, the Cherry3 camera (Cherry Imaging, Yokneam, Israel) is a new stereoscopic optical and high-resolution 3D imaging system that is specifically designed to objectively measure changes in above-surface scar volume after various interventions. It has been shown to be valid, accurate, and practical for assessing scar volume and for monitoring treatment response.[69]

- Skin specimens will be obtained via optional 2 mm punch biopsy on POD 0 (prior to surgery) and POD 30 (first follow-up visit after completion of LED-RL treatment). All optional skin biopsies will be perform by Dr. Daniel Siegel or Dr. Jared Jagdeo. Skin specimens will be used for histological analysis and molecular studies.
    - After skin biopsy, the sample will be placed in fixative (10% neutral buffered formalin) for routine tissue processing and embedding. Paraffin embedded sections will be mounted on glass slides and stained with hematoxylin and eosin (H&E) and either Masson's trichrome or picrosirius red for evaluation of collagen. Histological examination of skin specimens will be conducted to quantify the number of Ki-67 positive fibroblasts and to qualify collagen content.[77]
    - High-throughput assays will be performed using subject biopsy specimens to screen for molecular effects associated with LED-RL treatment. Molecular testing will include RNA-Seq, microRNA arrays, and quantitative real-time PCR (RT-PCR).
- Subjective scar evaluations using the Patient and Observer Scar Assessment Scale (POSAS) will be performed by the same blinded investigator (the treating surgeon) in conjunction with the subject at follow-up visits on POD 30, 90, and 180.
    - The POSAS was designed for a subjective evaluation of various types of scars. Van de Kar et al. critically tested the POSAS on linear scars and found it to have good internal consistency, reliability, and agreement.[58] Each subscale of the POSAS consists of six items rated on a numerical 10-point scale, with 10 indicating the "worst imaginable scar". The observer rates scar vascularity, pigmentation, thickness, relief, pliability, and surface area while the patient assesses pain, itching, color, stiffness, thickness, and relief. The scores of each of the six items are summed for a total score (range 6 to 60).

8.2 SAFETY AND OTHER ASSESSMENTS

Refer to *Section 1.3: Schedule of Activities*, for the sequence of events that should occur during the screening process.

Study Specific Procedures and Evaluations for Safety or Other Purposes

- The subject's medical history will be obtained by interview at the screening visit to screen for exclusion criteria.
- The subject's concomitant medication list will be reviewed at each study visit to screen for prohibited medications.
- A screening photosensitivity test will be conducted prior to enrollment, wherein the potential subject will be exposed to LED-RL for 20 minutes on the non-dominant upper forearm (as recommended by the manufacturer in the Omnilux new-U user guide), and then evaluated 24 hours later for evidence of photosensitivity. Criteria for photosensitivity include, but are not limited to: warmth, erythema, edema, blistering, rash, pain, or discomfort lasting more than 24 hours. If no adverse events occurred, the potential subject is deemed "non-photosensitive" and thus eligible for participation in the study.
- At the screening visit, the mini-facelift surgery will be discussed with the subject, including details of the procedure, risks, benefits, alternative options, expected outcomes, and pre- and post-operative instructions.
- The mini-facelift surgery will be performed in-office under local anesthesia by Dr. Daniel Siegel (co-investigator and board-certified dermatologic surgeon).
- A targeted physical examination of the treatment sites (i.e., the periauricular skin and surrounding areas) will be performed at each study visit.
- Each treatment session will be monitored for any safety issues or adverse events, either reported by the subject or observed by the investigator.
- Subjects will be provided with a daily diary to record any adverse events experienced at home during the 3-week treatment period. Investigators will call subjects on a weekly basis to monitor for adverse events during the same time period.
    - Common expected post-treatment effects of transient (i.e., lasts less than 24 hours) erythema, warmth, or edema at the treated sites will be recorded. However, any occurrence of these expected effects will not be considered an adverse event in safety data reports, unless the effect lasts more than 24 hours or is considered serious.
- An exit questionnaire will be administered at the final study visit to assess for patient-reported outcomes and elicit other feedback.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

In this study, there are predefined "common expected post-treatment effects" that should be transient (i.e., lasts less than 24 hours): erythema, warmth, and edema at the treated sites. These effects will be recorded on CRFs on safety monitoring, but will not be considered AEs unless the effect

persists longer than expected (i.e., does not resolve within 24 hours of treatment administration) or meets the definition of a serious AE.

Close attention will be paid to any incidences of cutaneous blistering at the treated sites, either within the incision sites or on the peri-incisional skin.

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, or a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All AEs must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or

other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the subject's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

If there is any doubt as to whether a clinical observation is an AE, the event will be reported. Evaluation of relatedness will consider etiologies such as natural history of the underlying disease, concurrent illness, concomitant therapy, study-related procedures, accidents, and other external factors.

8.3.3.3 EXPECTEDNESS

The PI will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to the study intervention (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The investigators will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

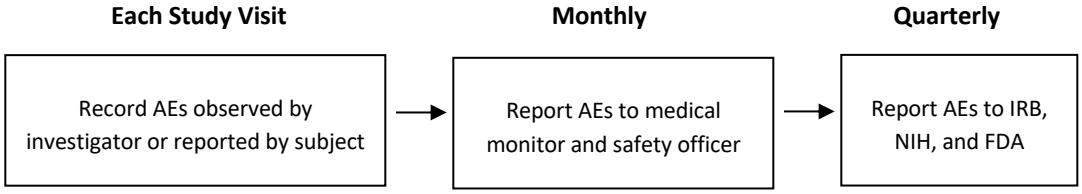
8.3.5 ADVERSE EVENT REPORTING

All required reportable events must be reported to the IRB within the deadlines specified in the SUNY Downstate Medical Center Policy IRB-01: Human Research Protections Program. Unanticipated adverse device effects (UADEs) will be reported as soon as possible, but no later than 10 days.

Any AEs that occur in-office during or immediately after treatment sessions, or reported by the subject in the AE diary or in weekly phone calls, will be captured on the appropriate CRF. Information to be collected includes a description of the AE, time of onset, severity, relationship to the study intervention, and time of resolution/stabilization.

Subjects will be counseled regarding the expected outcome of skin erythema and warmth, found to resolve within 24 hours in other clinical studies and occurs in 10% of participants.[70,71] For this study, the common expected post-treatment side effects of transient erythema, warmth, and swelling at the treated sites will be recorded, but will not be considered an AE for the purposes of safety reports.

All AEs will be reported to the designated medical monitor (Dr. Neil Brody), independent safety monitor (Dr. Jeannette Jakus), and internal faculty committee at monthly meetings. Please refer to *Section 10.1.6: Safety Oversight* for the composition of the internal committee. Dr. Jakus is the Director of Clinical Trials & Research in the Department of Dermatology at SUNY Downstate whose responsibilities include: managing and ensuring research compliance; monitoring all departmental clinical trials for AEs; and overseeing appropriate reporting of AEs to the IRB. AEs will be reported to the IRB, NIH, and FDA on a quarterly basis. The PI is responsible for completing and signing off on the AE reports.



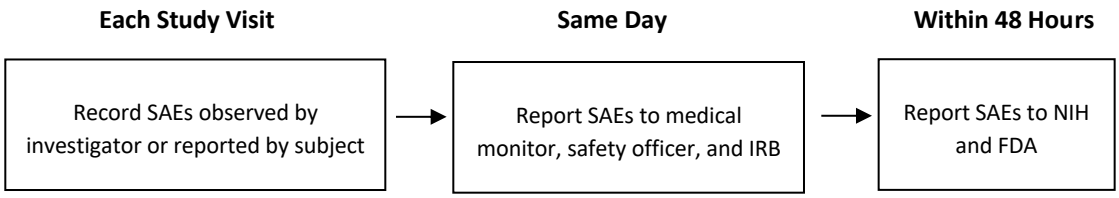
8.3.6 SERIOUS ADVERSE EVENT REPORTING

The study clinician will immediately report to the sponsor any SAE, whether or not considered study intervention related, including those listed in the protocol and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are SAEs must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event. In that case, the investigator must immediately report the event to the sponsor.

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the study sponsor and should be provided as soon as possible.

If applicable, the study investigator shall complete an Unanticipated Adverse Device Effect Form and submit to the study sponsor and to the reviewing IRB as soon as possible, but in no event later than 10 working days after the investigator first learns of the effect. The study sponsor is responsible for conducting an evaluation of an unanticipated adverse device effect and shall report the results of such evaluation to the FDA and to all reviewing IRBs and participating investigators within 10 working days after the sponsor first receives notice of the effect. Thereafter, the sponsor shall submit such additional reports concerning the effect as FDA requests.

Per SUNY Downstate policy for clinical trials, SAEs must be reported within 24 hours if an internal AE is serious, unanticipated, and would have implications for the conduct of the study. For this study, SAEs will be reported to the designated medical monitor (Dr. Neil Brody), safety officer (Dr. Jeannette Jakus) and the reviewing IRB on the same day of learning of the event. SAEs will be reported to the FDA and the NIH within 48 hours by express mail. The PI is responsible for completing and signing off on the SAE reports.



8.3.7 REPORTING EVENTS TO PARTICIPANTS

N/A

8.3.8 EVENTS OF SPECIAL INTEREST

N/A

8.3.9 REPORTING OF PREGNANCY

N/A

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems (UPs) involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This definition could include an unanticipated adverse device effect, any SAE on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report UPs to the reviewing IRB and to the PI. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are SAEs will be reported to the IRB on the same day of the investigator becoming aware of the event and to the study sponsor within 48 hours.
- Any other UP will be reported to the IRB and to the study sponsor within 10 working days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the OHRP within 10 working days of the IRB's receipt of the report of the problem from the investigator.

An investigator shall submit to the sponsor and to the reviewing IRB a report of any unanticipated adverse device effect occurring during an investigation as soon as possible, but in no event later than 10 working days after the investigator first learns of the effect (21 CFR 812.150(a)(1)). A sponsor who conducts an evaluation of an unanticipated adverse device effect under 812.46(b) shall report the results of such evaluation to the FDA and to all reviewing IRB's and participating investigators within 10 working days after the sponsor first receives notice of the effect. Thereafter the sponsor shall submit such additional reports concerning the effect as FDA requests (21 CFR 812.150(b)(1)).

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

N/A

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

- Hypothesis for Primary Efficacy Endpoint: There is a statistically significant difference in scar pliability between a LED-RL-treated incision and a mock-treated incision.
    - Skin elasticity and induration (an index of scar pliability) are measured pre-treatment at the first treatment session and post-treatment on follow-up visits at POD 30, 90, and 180.

- Hypothesis for Secondary Efficacy Endpoint(s): LED-RL phototherapy treatment results in a statistically significant improvement in POSAS score and VAS score compared to mock treatment.

9.2 SAMPLE SIZE DETERMINATION

The study will have three treatment arms (LED-RL 160 J/cm^2, LED-RL 320 J/cm^2, and LED-RL 480 J/cm^2) with 10 subjects each. The primary outcome measure is the difference in pliability between a treated incision site and the contralateral mock-treated incision site.

To calculate the sample size for this study, we anticipate that the minimal clinically important difference in scar pliability is 15%, based on minimum decrease in fibroblast cell number after LED-RL treatment from our *in vitro* data. A power analysis plan predicted that with 9 subjects, the study would have greater than 80% power to detect a 15% difference in pliability by using a two-sided paired *t*-test. To take into account a dropout rate of 10%, 10 subjects will be enrolled in each treatment arm. Up to 150 potential participants will be screened and a total of 30 subjects will be enrolled in the study.

9.3 POPULATIONS FOR ANALYSES

- Intention-to-treat (ITT) analysis dataset: all participants who are enrolled and randomly allocated to treatment, regardless of treatment actually receive

- Per-protocol analysis dataset: participants who adhered to the protocol and completed the treatment originally allocated (i.e., attended all treatment sessions)

- Safety analysis dataset: participants who received at least one dose of the study intervention

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

In initial intention-to-treat analyses, all available data will be included. Multiple imputation will be used where dependent variable values are missing. In subsequent per-protocol analyses, only subjects with complete data will be included.

SAS (SAS Institute, Cary NC) 9.4 statistical software will be used.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The two principal outcomes (scar pliability measurements of skin elasticity and skin induration) will be used separately as dependent variables (DVs) in mixed linear models. Fixed factors in each model will be treatment group, whether treated, side of face (left versus right), and time (3 points post-baseline). Baseline DV measure will be introduced as a scored covariate; subject ID as a random factor. The Akaike information criterion will be used to assess what intra-subject covariance structure might be optimal. Tests of Interaction among fixed factors will be conducted, and the utility of polynomial terms in the baseline DV investigated. Model residuals will be examined for skew and for outliers; the DV will be power-transformed if necessary to maximize normality of residuals.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

Secondary outcomes will be presented as descriptives tabulated by whether treated, treatment group and time, showing medians and ranges. No inferential analyses will be conducted, in order to minimize the multiple testing problem.

9.4.4 SAFETY ANALYSES

AEs, SAEs, and UPs will be recorded and reported as described in the protocol. The information documented about each event or problem includes onset, duration, severity, relationship to the study intervention, and outcome. Summary statistics of safety data will be presented as number of subjects who experienced AEs in each treatment group and a breakdown of the event types. AEs will be coded according to the terminology in the Medical Dictionary for Regulatory Activities (MeDRA). AEs leading to premature discontinuation from the study and serious treatment-emergent AEs will also be presented.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Descriptives of relevant subject characteristics (age, gender, race, and ethnicity) of the entire study population (i.e., intent-to-treat population) will be tabulated by treatment group. Continuous variables will be presented as median and range. Categorical variables will be presented as proportions. Significance tests will not be applied.

9.4.6 PLANNED INTERIM ANALYSES

N/A

9.4.7 SUB-GROUP ANALYSES

To determine if any differential effects of LED-RL are associated with demographics (race, ethnicity, age, and gender), a linear regression or robust linear regression with adjustment for treatment group effect on pliability will be performed.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Individual participant data will be listed by measure and time point for raw data analyses.

9.4.9 EXPLORATORY ANALYSES

N/A

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol:

- LED-Red Light Phototherapy for Skin Scarring Prevention Informed Consent for Research Study
- Consent to Invasive Procedure (Optional Skin Biopsy)
- Research Study Summary for Potential Participants

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants

will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the NIH. If the study is prematurely terminated or suspended, the PI will promptly inform study participants, the IRB, and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or FDA.

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsors and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be managed and protected in accordance with SUNY Downstate Medical Center Human Research Protections Program. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. Written study subject individual or aggregate data from the study will be secured in a locked cabinet, in a locked room. Computerized data will be stored on the HIPAA-compliant SUNY Downstate Department of Dermatology desktop computers that are connected to the SUNY Downstate Medical Center's secure network behind a firewall, complete with 128-bit data encryption. Storage of any PHI on a laptop or portable device (e.g., external drive, flash drive, CD/DVD, USB drive or similar) will be encrypted and used only for temporary storage. In addition to being encrypted, removable storage devices will be stored in a locked cabinet or drawer when not in use.

Certificate of Confidentiality

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Biological samples and data collected for this study will not be stored for future research after the study is completed.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	**Medical Monitor**
Jared Jagedo, MD, MS	Neil Brody, MD, PhD
SUNY Downstate Medical Center	SUNY Downstate Medical Center
Department of Dermatology 450 Clarkson Avenue Brooklyn, NY, 11203	Department of Dermatology 450 Clarkson Avenue Brooklyn, NY, 11203
917-837-9796	516-779-2377
jrjagdeo@gmail.com	neil.brody@downstate.edu

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of an Independent Safety Monitor (ISM) with the appropriate expertise. For this study, the ISM is Dr. Jeannette Jakus, the Director of Clinical Research in the Department of Dermatology at SUNY Downstate. The ISM is independent from the study conduct and free of conflict of interest. The ISM will meet at least semiannually to assess safety and efficacy data on each arm of the study. The primary responsibility of the ISM is to provide independent safety monitoring in a timely fashion, including by review of AEs, immediately after they occur or are reported, with follow-up through resolution. The ISM will provide her input to the PI and study sponsor.

For this study, monitoring will be performed on a quarterly basis by the following entities:

a. PI: Jared Jagdeo, MD, MS
b. SUNY Downstate IRB
c. Independent Safety Monitor: Jeannette Jakus, MD
d. Designated Medical Monitor: Neil Brody, MD, PhD
e. SUNY Downstate Department of Dermatology Clinical Research Faculty Committee: Neil Brody, MD, PhD; Edward Heilman, MD; Jeannette Jakus, MD

10.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with ICH GCP, and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by the designated ISM. Monitoring will be on-site throughout the study and includes targeted data verification of endpoint, safety and other key data variables.
- Each clinical site will perform internal quality management of study conduct, data collection, documentation and completion.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into REDCap (Research Electronic Data Capture), a 21 CFR Part 11-compliant data capture system provided by the SUNY Downstate Clinical & Translational Science Center. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.9.2 STUDY RECORDS RETENTION

Study documents will be securely maintained to protect confidentiality and privacy in accordance with Downstate policies. According to the minimum retention periods required by Downstate, research records collected by investigators will be maintained for at least three years, and up to 10 years as practicable, after completion of the research. Research participants' signed HIPAA Research Authorization forms will be kept for a minimum of six years after such authorization last was in effect. Research records will not be destroyed unless in conformity with Downstate policies and requirements of the NIH.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, ICH GCP, or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 10 working days of identification of the protocol deviation, or within 10 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to the NIGMS Program Official. Protocol deviations must be sent to the reviewing IRB per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIGMS has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ADDITIONAL CONSIDERATIONS

Scar Treatment

During the study, subjects are asked to refrain from using scar treatments to both periauricular incision sites, as to not confound the results of the study given that the objective is to evaluate clinical efficacy of LED-RL on scar reduction. Refer to *Section 2.3.3: Assessment of Potential Risks and Benefits* for a discussion of how prohibition of scar treatment during the study period does not pose significant harm to subjects. Briefly, patients undergoing facelift tend to have favorable wound healing outcomes (i.e., minimal scarring) since facial skin heals well after injury, and most surgical incisions ultimately heal with a normal scar.[41] Furthermore, the scar maturation process is lengthy (up to 2 years to produce the final scar result) and the vast majority of scars improve aesthetically over time as the fibrous scar tissue undergoes remodeling.[43] In other words, scars are not considered to be fully mature until 6 months-2 years after the injury. At 6 months post-surgery (coinciding with the final

study visit), scars will be evaluated to determine if they are pathological (e.g., hypertrophic scars or keloids) or appear consistent with normal healing. While scars can be associated with symptoms of pain and pruritus, scar treatment is considered cosmetic and elective in nature. Thus, prohibition of concomitant scar treatment or prophylaxis during the study is not expected to cause irreversible harm or suffering to subjects.

It is important to note that this stipulation should not be construed as subjects being "denied standard scar treatment" as 1) most scar treatments are not medically necessary, 2) it may take several months for pathological scarring to become evident (and the type of pathological scar guides clinical management), and 3) in clinical practice, many scars are observed during maturation and only treated if they become problematic to the patient. Also, in the postoperative period, the most important strategy to promote skin healing is proper wound care (i.e., maintenance of a clean and moist wound environment), which the subjects will be counseled on.

Subjects will be able to seek and receive elective scar treatments, if desired, at the conclusion of the study (i.e., after completion of the final study visit at approximately 6 months post-surgery). All subjects will be offered fractionated carbon dioxide (CO_2) laser treatment to the surgical incision sites for scar reduction. If a subject opts to have CO_2 laser resurfacing of the treated and/or untreated incision sites, the cost of the treatment will be covered by the Center for Photomedicine, Department of Dermatology. Laser resurfacing is an accepted therapeutic modality for scar revision that is commonly offered by dermatologists and has been shown to be effective in improving the appearance of various types of scars (e.g., traumatic, surgical, pathologic).[78–80] For management of post-facelift hypertrophic scars, it is preferred to wait at least 6 months following surgery to perform any scar revision procedures such as surgical revision, dermabrasion, and laser skin resurfacing.[46]

There is no "gold standard" for scar management and existing treatment options for scar reduction have variable efficacy in the evidence base. Current treatment options include silicone gels or sheets, intralesional corticosteroids, 5-fluorouracil, laser therapy, radiotherapy, cryotherapy, bleomycin, mitomycin C, imiquimod, pressure therapy, adhesive microporous hypoallergenic paper tape, onion extract, massage therapy, over-the-counter topical emollients for scars, laser therapy, and surgical revision.[76] No scar treatment can completely remove or eliminate scarring, only minimize its visibility and associated symptoms.

Limitations

There are limitations in this study due to the study design. In theoriginal study design, subjects are differentially randomized based on race and ethnicity, making skin color a confounding variable that cannot be controlled in the statistical analysis phase. That is, any observed clinical effects would have been difficult to interpret due to the possibility of differential effects of LED-RL phototherapy on different skin types. In the Phase I studies, our finding of differential safety profile of LED-RL based on skin type (i.e., skin of color individuals were more photosensitive and possibly at increased risk of blistering) was unexpected and not described previously.

In a joint discussion with Dr. Jeremy Weedon (biostatistician and Associate Director of the Scientific Computing Center at SUNY Downstate), we considered the following methodology changes and describe their advantages and disadvantages below.

1) Have two treatment groups, using only the low and medium intensity doses (Group 1 LED-RL 160 J/cm^2 and Group 2 LED-RL 320 J/cm^2).

a. Advantages: Since the stratification of treatment dose based on skin type is removed, all subjects can be randomized to Group 1 or Group 2. The groups may be more balanced in terms of baseline characteristics. With a larger sample size of n=15 in each group, there is greater power to the study.
b. Disadvantages: Based on *in vitro* data, LED-RL phototherapy as antifibrotic effects on skin fibroblasts at fluences of 320 J/cm^2 and above. The clinical efficacy of LED-RL to treat skin fibrosis is unknown and a wide dose-ranging study is necessary to discern any therapeutic effects at different fluences. Exclusion of Group 3 (LED-RL 480 J/cm^2) from the study may limit our ability to detect these effects. For example, there may be no clinical difference between the treated and untreated scars at 320 J/cm^2, but a difference may be apparent at 480 J/cm^2.

2) Enroll only Caucasian non-Hispanic subjects and keep all three treatment groups.
   a. Advantages: The block randomization process will be simplified since there is no need to consider skin type and safety of certain LED-RL fluences. Also, since the subject population is homogeneous in terms of race and ethnicity, any observed clinical effects of LED-RL (e.g., significant difference in primary outcome between a treated and untreated scar at a certain treatment dose) can be interpreted as an effect of the study treatment without the confounding variable of skin type.
   b. Disadvantages: Under the current inclusion criteria, individuals of any race and ethnicity may be eligible to enroll in the study. We do not want to exclude any potential subjects based on skin color. Racial and ethnic minorities are underrepresented in clinical trials and the NIH Revitalization Act of 1993 mandates the appropriate inclusion of minorities in all NIH-funded research.[81,82] In addition, given the demographics of the patient population at SUNY Downstate, a targeted study with only Caucasian non-Hispanic individuals would exclude many potential study participants from this population.

3) Enroll subjects of any race/ethnicity and randomize them to all three treatment groups, with the understanding that safety data from Phase I studies suggest that skin of color subjects are more susceptible to blistering at higher fluences.
   a. Advantages: The block randomization process will be simplified if skin of color subjects are not excluded from the highest intensity treatment group. The groups may be more balanced in terms of baseline characteristics.
   b. Disadvantages: Based upon safety data generated from our two Phase I studies, we concluded that the maximum tolerated dose (MTD) of LED-RL phototherapy is 320 J/cm^2 for skin of color subjects. This MTD was established as a precaution after one African-American subject developed a small skin blister at the treatment site after a LED-RL phototherapy session administered at 480 J/cm^2. While we cannot make any inferences about the true rate of this adverse event in the skin of color population, we do not want to enroll these subjects into the highest intensity treatment group knowing that the risk of blistering may be greater.

4) Conduct two separate clinical trials – one with skin of color subjects only and the other one with Caucasian non-Hispanic subjects only.
   a. Advantages: The block randomization process will be simplified in each trial. Any observed clinical effects of LED-RL can be interpreted in the context of skin type,

without having to consider the possibility of differential therapeutic efficacy based on race and ethnicity.

b. Disadvantages: This approach has the same limitations as option #3, plus would be challenging to implement in terms of logistics and feasibility. Conducting two clinical trials would require doubling the sample size (i.e., enroll a total of 60 subjects) to achieve statistical power, and it would be logistically difficult to recruit for a study of this size.

In a joint discussion with the IRB, this version of the protocol was modified to reflect a change in the study design to reflect option #3 above. It was agreed that the unequal randomization scheme of the original study design (i.e., participants would undergo different randomization algorithms based on race/ethnicity, as skin of color individuals are restricted from receiving the highest intensity dose) would be a major potential confounding factor during data analysis. In this protocol, all subjects of any race/ethnicity will undergo a single randomization process to be allocated to any 1 of the 3 treatment groups. That is, subjects are randomized equally without regard to skin type. This change in the study design was made after carefully considering the risks and benefits of allowing skin of color subjects to receive the highest treatment intensity dose.

Based on the safety data from the Phase I trials described above, we believe skin of color individuals are more photosensitive to LED-RL phototherapy and are therefore at increased risk of adverse events such as blister formation at higher intensity doses. However, this hypothesis is based on a single observation that one African-American subject developed a small skin blister at the treatment site after a LED-RL phototherapy session administered at 480 J/cm^2. Therefore, the MTD of 320 J/cm^2 for skin of color individuals is a precautionary determination, as no definitive conclusions can be made about LED-RL safety from a limited sample size. Further evaluation of safety is required, especially in regards to skin of color subjects who receive LED-RL at a fluence of 480 J/cm^2. By allowing skin of color subjects to be enrolled in the highest treatment intensity group, we will be able to generate more safety data with a larger sample size, as well as interpret any statistically significant clinical effects more accurately (without skin type as a confounding variable).

During the informed consent process, potential subjects will be counseled extensively on the known potential risks of LED-RL phototherapy. All potential subjects will be informed verbally and in writing (included in the consent form) that previous safety data suggest a higher risk of blistering in individuals with skin of color.

10.3 ABBREVIATIONS

AE	Adverse Event
ANOVA	Analysis of Variance
bFGF	Basic Fibroblast Growth Factor
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CTGF	Connective Tissue Growth Factor
DHHS	Department of Health and Human Services

DSMB	Data Safety Monitoring Board
DV	Dependent Variable
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
H&E	Hematoxylin and Eosin
HIPAA	Health Insurance Portability and Accountability Act
ICC	Intraclass Correlation Coefficient
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITT	Intention-To-Treat
LED-RL	Light Emitting Diode-Red Light
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MRSD	Maximum Recommended Starting Dose
MTD	Maximum Tolerated Dose
MSDS	Material Safety Data Sheet
NIGMS	National Institute of General Medical Sciences
NIH	National Institutes of Health
OCT	Optical Coherence Tomography
OHRP	Office for Human Research Protections
OSAS	Observer Scar Assessment Scale
PDGF	Platelet-Derived Growth Factor
PHI	Protected Health Information
PI	Principal Investigator
POSAS	Patient and Observer Scar Assessment Scale
PSAS	Patient Scar Assessment Scale
qRT-PCR	Quantitative Reverse Transcription Polymerase Chain Reaction
QC	Quality Control
REDCap	Research Electronic Data Capture
ROS	Reactive Oxygen Species
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMAS	Superficial Musculoaponeurotic System
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOP	Standard Operating Procedure
TGF-β	Transforming Growth Factor-Beta
UP	Unanticipated Problem
US	United States
UV	Ultraviolet
VAS	Visual Analogue Scale

10.4 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale

11 REFERENCES

1. Longaker MT, Rohrich RJ, Greenberg L, et al. A randomized controlled trial of the embrace advanced scar therapy device to reduce incisional scar formation. Plast Reconstr Surg 2014;134(3):536–46.
2. Karmisholt KE, Taudorf EH, Wulff CB, Wenande E, Philipsen PA, Haedersdal M. Fractional CO2 laser treatment of caesarean section scars—A randomized controlled split-scar trial with long term follow-up assessment. Lasers Surg Med 2017;49(2):189–97.
3. Lev-Tov H, Mamalis A, Brody N, Siegel D, Jagdeo J. Inhibition of fibroblast proliferation in vitro using red light-emitting diodes. Dermatologic Surg 2013;39(8):1167–70.
4. Mamalis A, Jagdeo J. Light-emitting diode-generated red light inhibits keloid fibroblast proliferation. Dermatologic Surg 2015;41(1):35–9.
5. Brown BC, McKenna SP, Siddhi K, McGrouther DA, Bayat A. The hidden cost of skin scars: quality of life after skin scarring. J Plast Reconstr Aesthetic Surg 2008;61(9):1049–58.
6. Bayat A, McGrouther D, Ferguson M. Skin scarring. BMJ 2003;326(7380):88–92.
7. Bock O, Schmid-Ott G, Malewski P, Mrowietz U. Quality of life of patients with keloid and hypertrophic scarring. Arch Dermatol Res 2006;297(10):433–8.
8. Custis T, Armstrong AW, King TH, Sharon VR, Eisen DB. Effect of Adhesive Strips and Dermal Sutures vs Dermal Sutures Only on Wound Closure: A Randomized Clinical Trial. JAMA Dermatology 2015;151(8):862–7.
9. Gurtner GC. Discussion: No Efficacy for Silicone Gel Sheeting in Prevention of Abnormal Scar Formation in Children with Cancer: A Randomized Controlled Trial. Plast Reconstr Surg 2015;135(4):1095–6.
10. Braam KI, Kooijmans ECM, Van Dulmen-Den Broeder E, et al. No efficacy for silicone gel sheeting in prevention of abnormal scar formation in children with cancer: A randomized controlled trial. Plast Reconstr Surg 2015;135(4):1086–94.
11. Walmsley GG, Maan ZN, Wong VW, et al. Scarless wound healing: Chasing the holy grail. Plast Reconstr Surg 2015;135(3):907–17.
12. Wong VW, You F, Januszyk M, Gurtner GC, Kuang AA. Transcriptional profiling of rapamycin-treated fibroblasts from hypertrophic and keloid scars. Ann Plast Surg 2014;72(SUPPL. 2):711–9.
13. Liebel F, Kaur S, Ruvolo E, Kollias N, Southall MD. Irradiation of skin with visible light induces reactive oxygen species and matrix-degrading enzymes. J Invest Dermatol 2012;132(7):1901–7.
14. Sakamoto F, Avram M AR. Chapter 136: Lasers and Other Energy Technologies - Principles & Skin Interactions. In: Bolognia J, Jorrizo J, Schaffer J, editors. Dermatology. 2012.
15. Jagdeo JR, Adams LE, Brody NI, Siegel DM. Transcranial Red and Near Infrared Light Transmission in a Cadaveric Model. PLoS One 2012;7(10):1–10.
16. Campbell SM, Tyrrell J, Marshall R, Curnow A. Effect of MAL-photodynamic therapy on hypertrophic scarring. Photodiagnosis Photodyn Ther 2010;7(3):183–8.
17. Nie Z, Bayat A, Behzad F, Rhodes LE. Positive response of a recurrent keloid scar to topical methyl aminolevulinate-photodynamic therapy. Photodermatol Photoimmunol Photomed 2010;26(6):330–2.
18. Sakamoto FH, Izikson L, Tannous Z, Zurakowski D, Anderson RR. Surgical scar remodelling after photodynamic therapy using aminolaevulinic acid or its methylester: A retrospective, blinded study of patients with field cancerization. Br J Dermatol 2012;166(2):413–6.
19. Hession MT, Markova A, Graber EM. A review of hand-held, home-use cosmetic laser and light devices. Dermatologic Surg 2015;41(3):307–20.

20. Chung HJ, Steplewski A, Chung KY, Uitto J, Fertala A. Collagen fibril formation: A new target to limit fibrosis. J Biol Chem 2008;283(38):25879–86.
21. Wu CS, Wu PH, Fang AH, Lan CCE. FK506 inhibits the enhancing effects of transforming growth factor (TGF)-β1 on collagen expression and TGF-β/Smad signalling in keloid fibroblasts: Implication for new therapeutic approach. Br J Dermatol 2012;167(3):532–41.
22. Wang H, Zhuang K, Gao L, Zhang L, Yang H. Increased expression of CCN2 in the red flashing light-induced myopia in guinea pigs. Biomed Res Int 2013;2013.
23. Seo YK, Park JK, Song C, Kwon SY. Comparison of light-emitting diode wavelength on activity and migration of rabbit ACL cells. Lasers Med Sci 2014;29(1):245–55.
24. de Vasconcelos Catão MHC, Nonaka CFW, de Albuquerque RLC, Bento PM, de Oliveira Costa R. Effects of red laser, infrared, photodynamic therapy, and green LED on the healing process of third-degree burns: clinical and histological study in rats. Lasers Med Sci 2014;30(1):421–8.
25. Karu TI. Multiple roles of cytochrome c oxidase in mammalian cells under action of red and IR-A radiation. IUBMB Life 2010;62(8):607–10.
26. Ghahary A, Tredget EE, Ghahary A, Bahar MA, Telasky C. Cell proliferating effect of latent transforming growth factor-beta1 is cell membrane dependent. Wound Repair Regen 2002;10(1067–1927):328–35.
27. M.H.Barcellos-Hoff, A.Dix T. Redox-mediated activation of latent transforming growth factor-beta1. Mol Endocrinol 1996;10(9):1077–83.
28. Cutroneo KR. TGF-beta-induced fibrosis and SMAD signaling: Oligo decoys as natural therapeutics for inhibition of tissue fibrosis and scarring. Wound Repair Regen 2007;15(SUPPL. 1).
29. Jobling MF, Mott JD, Finnegan MT, et al. Isoform-Specific Activation of Latent Transforming Growth Factor β (LTGF-β) by Reactive Oxygen Species. Radiat Res 2006;166(6):839–48.
30. Lorda-Diez CI, Montero JA, Garcia-Porrero JA, Hurle JM. Tgf2 and 3 are coexpressed with their extracellular regulator Ltbp1 in the early limb bud and modulate mesodermal outgrowth and BMP signaling in chicken embryos. BMC Dev Biol 2010;10.
31. Arany P. Photobiomodulation by Low Power Laser Irradiation Involves Activation of Latent TGF-β1. In: Waynant R, Tata D, editors. Proceedings of Light-Activated Tissue Regeneration and Therapy Conference. 2008. p. 207–12.
32. Massague J. TGFbeta Signaling: Receptors, Transducers, and Mad Proteins. Cell 1996;85:947–50.
33. Chin GS, Liu W, Peled Z, et al. Differential expression of transforming growth factor-beta receptors I and II and activation of Smad 3 in keloid fibroblasts. Plast Reconstr Surg 2001;108(2):423–9.
34. Yan X, Liu Z, Chen Y. Regulation of TGF-beta signaling by Smad7. Acta Biochim Biophys Sin (Shanghai) 2009;41(4):263–72.
35. Penn JW, Grobbelaar AO, Rolfe KJ. The role of the TGF-β family in wound healing, burns and scarring: a review. Int J Burns Trauma 2012;2(1):18–28.
36. Khoo YT, Ong CT, Mukhopadhyay A, et al. Upregulation of secretory connective tissue growth factor (CTGF) in keratinocyte-fibroblast coculture contributes to keloid pathogenesis. J Cell Physiol 2006;208(2):336–43.
37. Babalola O, Mamalis A, Lev-Tov H, Jagdeo J. The role of microRNAs in skin fibrosis. Arch Dermatol Res 2013;305(9):763–76.
38. Azzi L, El-Alfy M, Martel C, Labrie F. Gender differences in mouse skin morphology and specific effects of sex steroids and dehydroepiandrosterone. J Invest Dermatol 2005;124(1):22–7.
39. Gupta V, Winocour J, Shi H, Shack RB, Grotting JC, Higdon KK. Preoperative Risk Factors and Complication Rates in Facelift: Analysis of 11,300 Patients. Aesthetic Surg J 2015;36(1):1–13.

40. Aoki M, Akaishi S, Matsumoto NM, et al. Periauricular Keloids on Face-Lift Scars in a Patient with Facial Nerve Paralysis. Plast Reconstr Surg - Glob Open 2017;5(7):1–3.
41. Son D, Harijan A. Overview of surgical scar prevention and management. J Korean Med Sci 2014;29(6):751–7.
42. van den Broek LJ, Limandjaja GC, Niessen FB, Gibbs S. Human hypertrophic and keloid scar models: principles, limitations and future challenges from a tissue engineering perspective. Exp Dermatol 2014;23(6):382–6.
43. Gold MH, McGuire M, Mustoe TA, et al. Updated international clinical recommendations on scar management: Part 2 - Algorithms for scar prevention and treatment. Dermatologic Surg 2014;40(8):825–31.
44. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature 2008;453(7193):314–21.
45. Marshall CD, Hu MS, Leavitt T, Barnes LA, Lorenz HP, Longaker MT. Cutaneous Scarring: Basic Science, Current Treatments, and Future Directions. Adv Wound Care [Internet] 2018;7(2):29–45. Available from: http://online.liebertpub.com/doi/10.1089/wound.2016.0696
46. Bloom JD, Immerman SB, Rosenberg DB. Face-lift complications. Facial Plast Surg 2012;28(3):260–72.
47. Enomoto DNH, Mekkes JR, Bossuyt PMM, Hoekzema R, Bos JD. Quantification of cutaneous sclerosis with a skin elasticity meter in patients with generalized scleroderma. J Am Acad Dermatol 1996;35(3):381–7.
48. Draaijers LJ, Botman YAM, Tempelman FRH, Kreis RW, Middelkoop E, Van Zuijlen PPM. Skin elasticity meter or subjective evaluation in scars: A reliability assessment. Burns 2004;30(2):109–14.
49. Fong SS, Hung LK, Cheng JC. The cutometer and ultrasonography in the assessment of postburn hypertrophic scar--a preliminary study. Burns 1997;23 Suppl 1(8):S12-8.
50. Falanga V, Bucalo B. Use of a durometer to assess skin hardness. J Am Acad Dermatol 1993;29(1):47–51.
51. Magliaro A, Romanelli M. Skin hardness measurement in hypertrophic scars. Wounds 2003;15(3):66–70.
52. Taylor B, McGrouther DA, Bayat A. Use of a non-contact 3D digitiser to measure the volume of keloid scars: a useful tool for scar assessment. J Plast Reconstr Aesthetic Surg 2007;60(1):87–94.
53. Fearmonti R, Bond J, Erdmann D, Levinson H. A review of scar scales and scar measuring devices. J Plast Surg 2010;10:e43.
54. Virén T, Iivarinen JT, Sarin JK, Harvima I, Mayrovitz HN. Accuracy and reliability of a hand-held in vivo skin indentation device to assess skin elasticity. Int J Cosmet Sci 2018;40(2):134–40.
55. Sun D, Yu Z, Chen J, Wang L, Han L, Liu N. The Value of Using a SkinFibroMeter for Diagnosis and Assessment of Secondary Lymphedema and Associated Fibrosis of Lower Limb Skin. Lymphat Res Biol 2017;15(1):70–6.
56. Kim MA, Kim EJ, Lee HK. Use of SkinFibrometer® to measure skin elasticity and its correlation with Cutometer® and DUB® Skinscanner. Ski Res Technol 2018;(January):466–71.
57. Mamalis A, Koo E, Garcha M, Murphy WJ, Isseroff RR, Jagdeo J. High fluence light emitting diode-generated red light modulates characteristics associated with skin fibrosis. J Biophotonics 2016;9(11–12):1167–79.
58. Van De Kar AL, Corion LUM, Smeulders MJC, Draaijers LJ, Van Der Horst CMAM, Van Zuijlen PPM. Reliable and feasible evaluation of linear scars by the patient and observer scar assessment scale. Plast Reconstr Surg 2005;116(2):514–22.
59. Truong PT, Lee JC, Soer B, Gaul CA, Olivotto IA. Reliability and validity testing of the patient and

observer scar assessment scale in evaluating linear scars after breast cancer surgery. Plast Reconstr Surg 2007;119(2):487–94.
60. Draaijers LJ, Tempelman FRH, Botman YAM, et al. The Patient and Observer Scar Assessment Scale: A reliable and feasible tool for scar evaluation. Plast Reconstr Surg 2004;113(7):1960–5.
61. Bae SH, Bae YC. Analysis of frequency of use of different scar assessment scales based on the scar condition and treatment method. Arch Plast Surg 2014;41(2):111–5.
62. Duncan JAL, Bond JS, Mason T, et al. Visual analogue scale scoring and ranking: A suitable and sensitive method for assessing scar quality? Plast Reconstr Surg 2006;118(4):909–18.
63. Micomonaco DC, Fung K, Mount G, et al. Development of a new visual analogue scale for the assessment of area scars. J Otolaryngol - Head Neck Surg 2009;38(1):77–89.
64. Abignano G, Aydin SZ, Castillo-Gallego C, et al. Virtual skin biopsy by optical coherence tomography: The first quantitative imaging biomarker for scleroderma. Ann Rheum Dis 2013;72(11):1845–51.
65. Babalola O, Mamalis A, Lev-Tov H, Jagdeo J. Optical coherence tomography (OCT) of collagen in normal skin and skin fibrosis. Arch Dermatol Res 2014;306(1):1–9.
66. Greaves NS, Benatar B, Whiteside S, Alonso-Rasgado T, Baguneid M, Bayat A. Optical coherence tomography: A reliable alternative to invasive histological assessment of acute wound healing in human skin? Br J Dermatol 2014;170(4):840–50.
67. Zhang N, Shi K, Hong L, Zhao J, Yu J. Antera 3D camera: A novel method for evaluating the therapeutic efficacy of fractional CO 2 laser for surgical incision scars. J Cosmet Dermatol 2018;(June):1–5.
68. Linming F, Wei H, Anqi L, et al. Comparison of two skin imaging analysis instruments: The VISIA®from Canfield vs the ANTERA 3D®CS from Miravex. Ski Res Technol 2018;24(1):3–8.
69. Salameh F, Koren A, Sprecher E, Artzi O. Novel Stereoscopic Optical System for Objectively Measuring Above-Surface Scar Volume—First-Time Quantification of Responses to Various Treatment Modalities. Dermatologic Surg [Internet] 2018;44(6):848–54. Available from: http://insights.ovid.com/crossref?an=00042728-201806000-00013
70. Sadick NS. A study to determine the efficacy of a novel handheld light-emitting diode device in the treatment of photoaged skin. J Cosmet Dermatol 2008;7(4):263–7.
71. Sadick NS. Handheld LED array device in the treatment of acne vulgaris. J Drugs Dermatol 2008;7(4):347–50.
72. Racial and Ethnic Categories and Definitions for NIH Diversity Programs and for Other Reporting Purposes. 2015;
73. Wainwright NJ, Dawe RS, Ferguson J. Narrowband ultraviolet B (TL-01) phototherapy for psoriasis: Which incremental regimen? Br J Dermatol 1998;139(3):410–4.
74. Calzavara-Pinton PG, Sala R, Arisi M, Rossi MT, Venturini M, Ortel B. Synergism between narrowband ultraviolet B phototherapy and etanercept for the treatment of plaque-type psoriasis. Br J Dermatol 2013;169(1):130–6.
75. Anderson KL, Feldman SR. A guide to prescribing home phototherapy for patients with psoriasis: The appropriate patient, the type of unit, the treatment regimen, and the potential obstacles. J Am Acad Dermatol 2015;72(5):868–78.
76. Gold MH, Berman B, Clementoni MT, Gauglitz GG, Nahai F, Murcia C. Updated international clinical recommendations on scar management: Part 1 - Evaluating the evidence. Dermatologic Surg 2014;40(8):817–24.
77. Cuttle L, Nataatmadja M, Fraser JF, Kempf M, Kimble RM, Hayes MT. Collagen in the scarless fetal skin wound: Detection with Picrosirius-polarization. Wound Repair Regen 2005;13(2):198–204.
78. Jagdeo J, Shumaker PR. Traumatic Scarring. JAMA Dermatology 2017;153(3):364.

79. Kraeva E, Ho D, Jagdeo J, Ms M. Successful Treatment of Keloid With Fractionated Carbon Dioxide (CO2) Laser and Laser-Assisted Drug Delivery of Triamcinolone Acetonide Ointment in an African-American Man. J Drugs Dermatol 2017;16(9):925–7.
80. Ho D, Jagdeo J. Excellent aesthetic and functional outcome after fractionated carbon dioxide laser skin graft revision surgery: case report and review of laser skin graft revision techniques. J Drugs Dermatology 2015;14(11):1285–8.
81. Chen MS, Lara PN, Dang JHT, Paterniti DA, Kelly K. Twenty years post-NIH Revitalization Act: Enhancing minority participation in clinical trials (EMPaCT): Laying the groundwork for improving minority clinical trial accrual. Cancer 2014;120(0 7):1091–6.
82. Hamel LM, Penner LA, Albrecht TL, Heath E, Gwede CK, Eggly S. Barriers to Clinical Trial Enrollment in Racial and Ethnic Minority Patients With Cancer. Cancer Control 2016;23(4):327–37.