NCT04834817 Effect of Combined Red and Near Infrared Light-emitting Diode (LED) Therapy on Tissue Regeneration Post Laser Treatment (Document date: 11/29/2023)

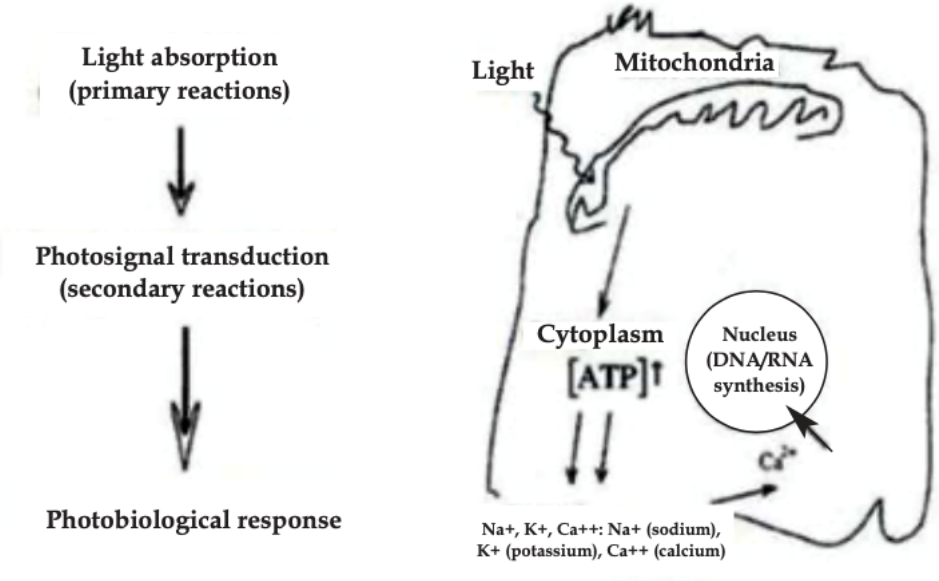
STUDY PROTOCOL

Background & Purpose of the Research

The objective of this study is to evaluate the effect of a combination of near infrared (830 nm) red (633 nm) and blue (465nm) light-emitting diode (LED) therapy on wound healing after application of low density ablative fractional laser to bilateral inner biceps, close to the axilla. Our hypothesis is that the LED therapy will result in less post treatment pain and in a faster healing time after ablative fractional laser application.

Literature reveals that light-emitting diodes (LED) promote healing of skin wounds by decreasing inflammation, increasing fibroblast proliferation, stimulating angiogenesis, formation of granulation tissue, and increasing collagen synthesis.1 LED treatment is of interest to the medical community in large part for its safety profile – at 6.5 milliwatts, it emits energy far less than that of a household flashlight. To further determine the extent to which LED promotes healing and to evaluate the relative speed at which it does so compared to other conservative management, this study sets out to assess wound healing with LED treatment after application of fractional ablative laser to the bilateral inner biceps, close to the underarm. Ablative fractional laser treatment results in well-healed regeneration of the superficial layers of the skin, thus an ablative fractional laser with the following parameters will be used to induce a two-inch by two-inch, superficial wound on each ventral bicep: 650 micrometers at 150 J and 5.5% density, similar to parameters used to treat scars. After laser application, we will evaluate the effect of a combination of near infrared (830 nm) red (633 nm) and blue (465nm) light-emitting diode (LED) therapy on wound healing post laser treatment. Product under investigation: BioPhotas Celluma LED device Technical parameters Red: 640nm+/-25nm NIR: 880nm+/-50nm Blue 465nm Irradiance 6.5 mW/cm2 Fluence 11.7J/cm2

Literature reveals that light-emitting diodes (LED) promotes healing of skin wounds by decreasing inflammation, increasing fibroblast proliferation, stimulating angiogenesis, formation of granulation tissue, and increasing collagen synthesis. One proposed mechanism for these changes called the Karu Model is shown below (Figure 1):



Source: Huang YY, et al. 2009.

Figure 1. Karu Model

Many studies have demonstrated these effects on wound healing in vitro, as below, where "LLLT" refers to low-level light therapy. See Figures 2 & 3 below.

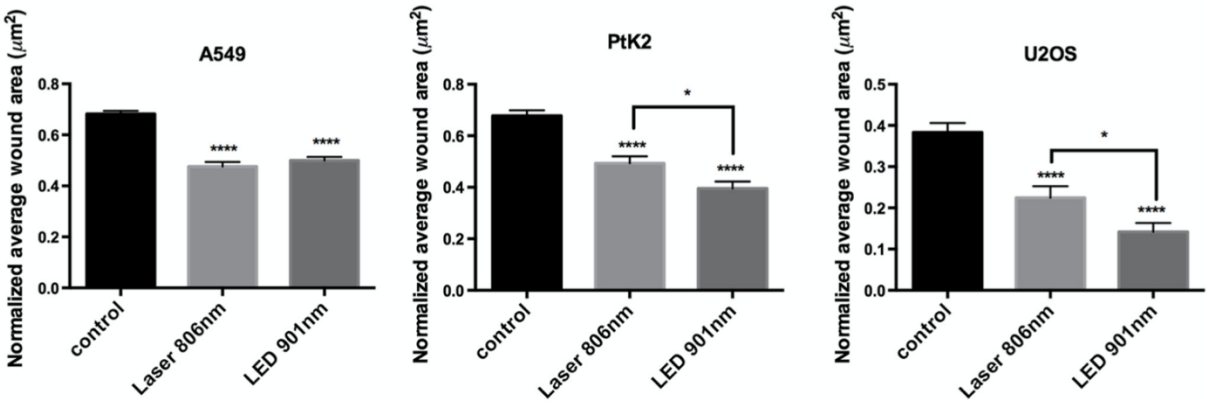
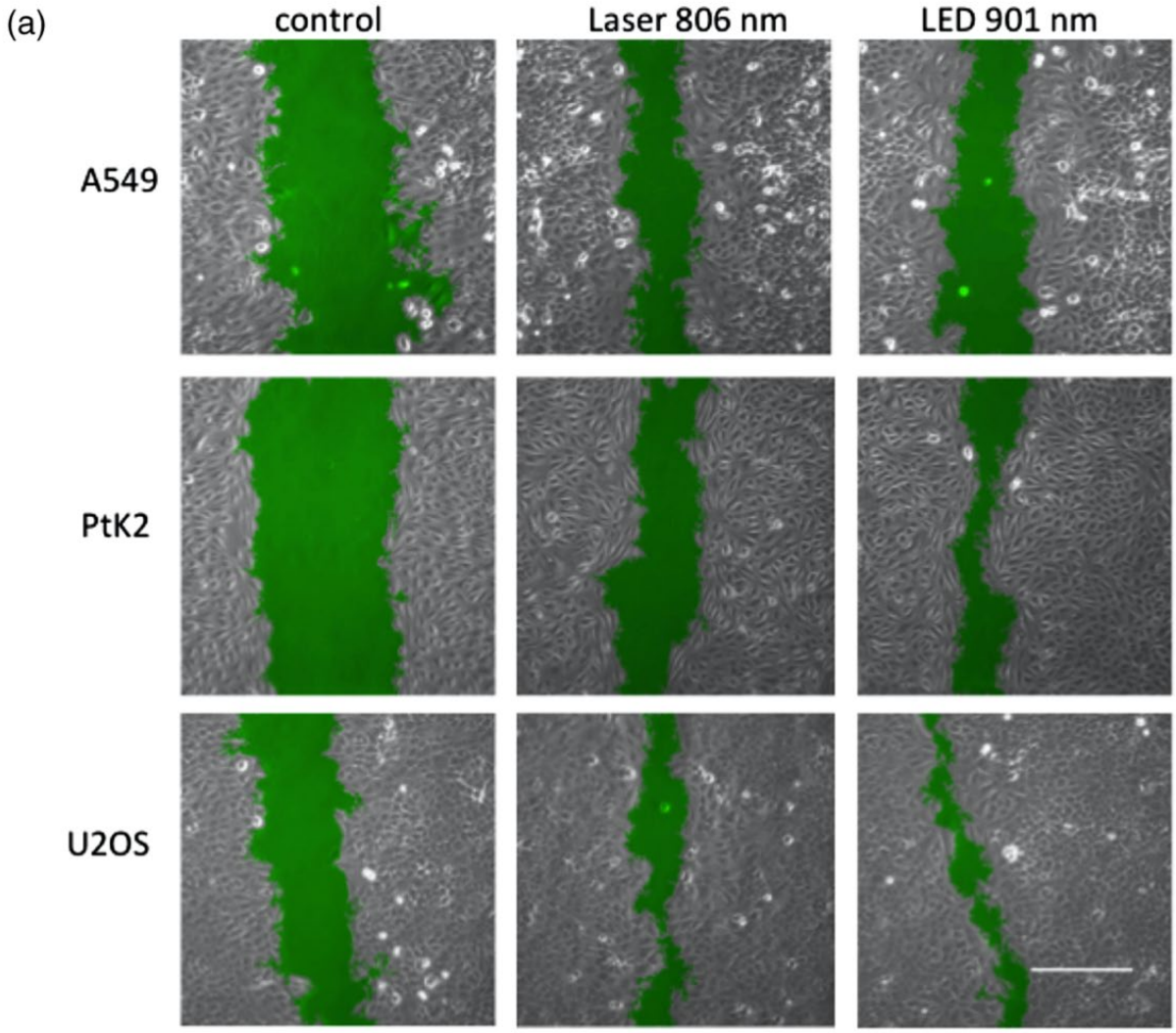


Fig. 3 LLLT increases *in vitro* wound healing in A549, PtK2, and U2OS using wavelengths 806 and 901 nm. (a and b) Cells were treated and evaluated as in (Fig. 2), but treated with LLLT 806 or 901 nm and wound healing was measured after 24 h. The green area marks the wound, bar, 100 μm. Data represent the mean $\pm$ SEM of at least three separate experiments with $N = 12$; ****$p < 0.0001$, *$p < 0.05$ compared with control or for the indicated comparison.



Nasa has demonstrated reproducible results here. See Figure 4, 6, and 7.

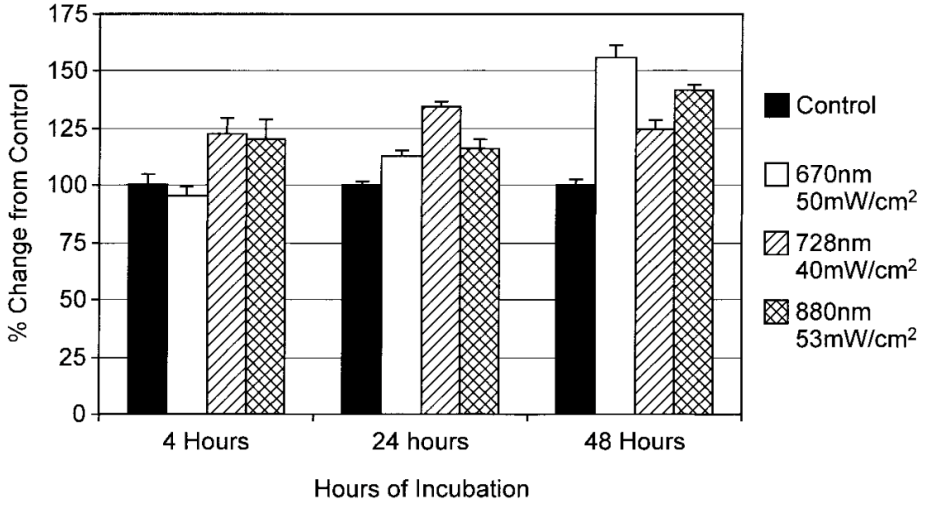


FIG. 4. LED response at 4 J/cm^2, 50 mW/cm^2 using individual wavelengths of 670, 728, and 880 nm (percentage change from control versus number of hours after LED treatment).

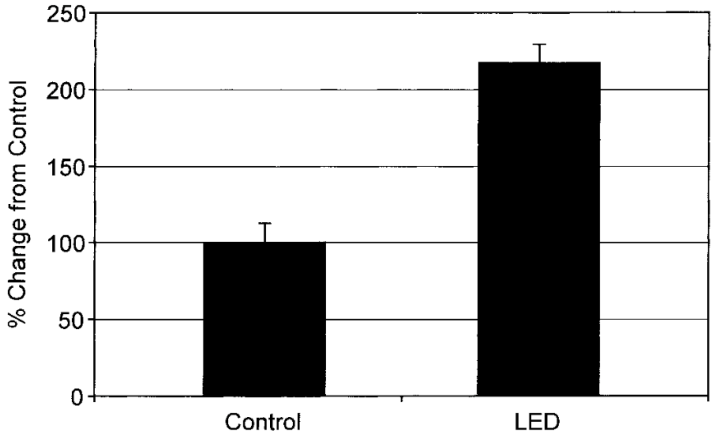


FIG. 6. HaCAT epithelial cell collagen synthesis at 8 J/cm^2, 50 mW/cm^2, 670 nm. Shown in 24-h ^{3}H proline incorporation.

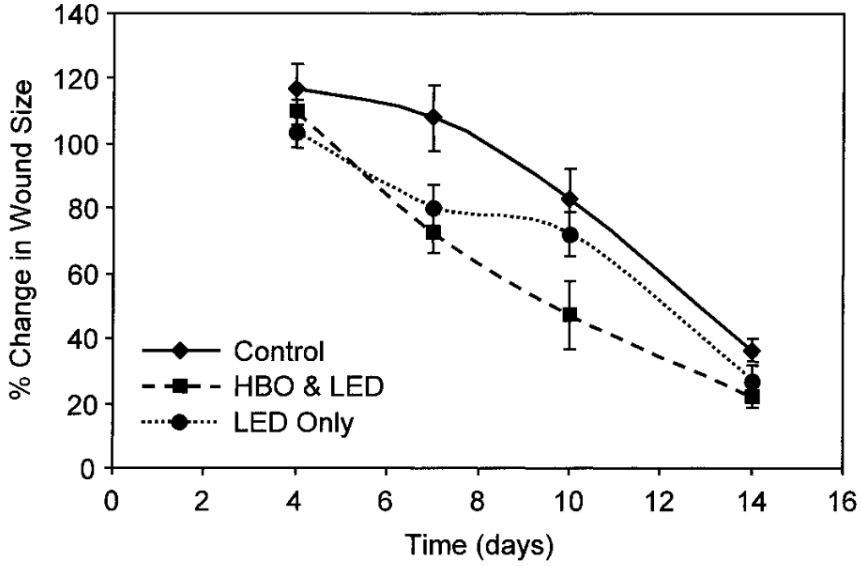


FIG. 7. Change in wound size in rat ischemic wound model versus time (days).

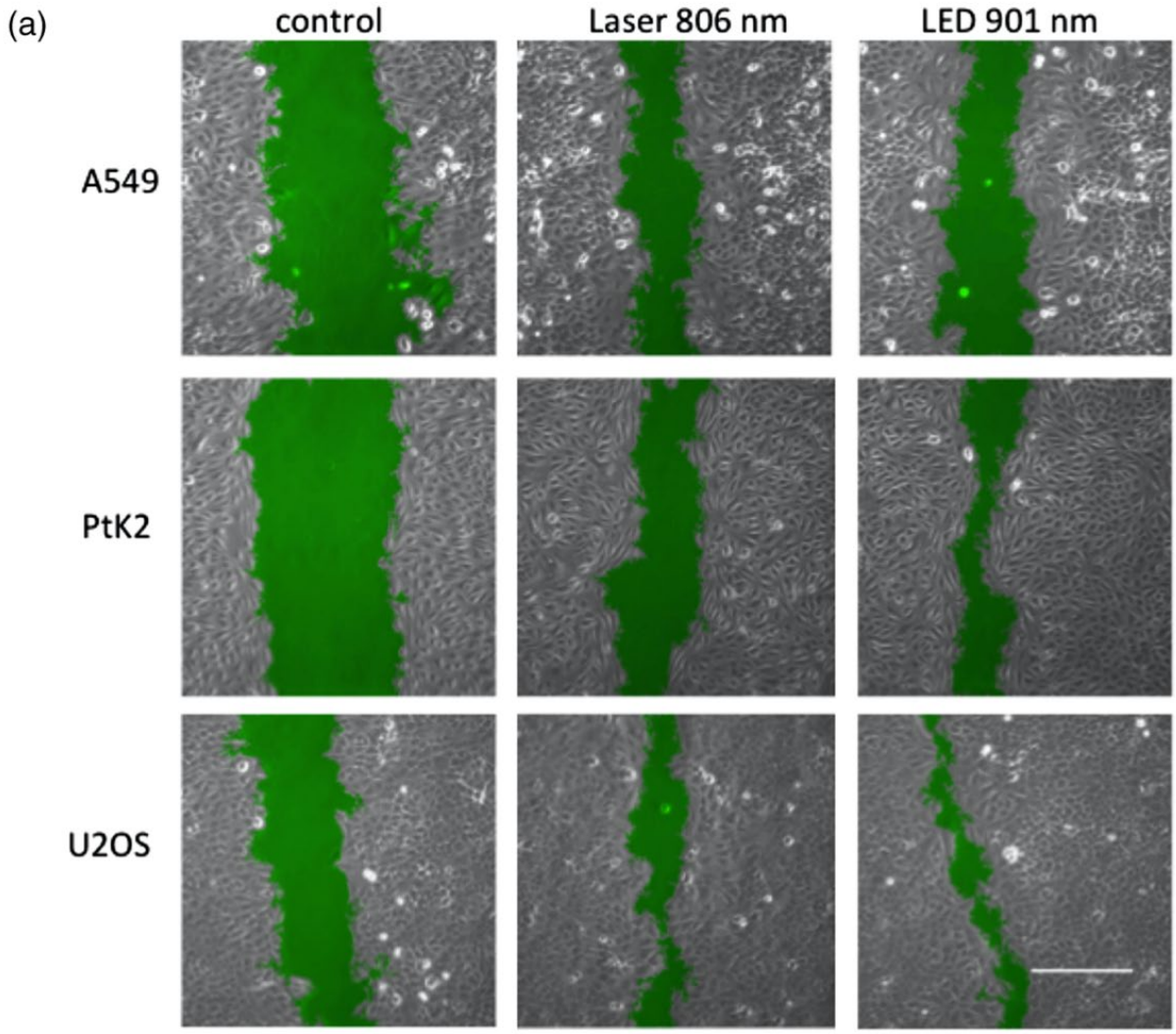
There is also evidence that LED treatment post-sternotomy leads to a decrease in bleeding and dehiscence, while providing an analgesic effect.

Visible LED light treatment at various wavelengths have been shown to not only increase growth in multiple cell lines but also to accelerate wound healing in human studies for skin grafts, chronic leg ulcers, and post-CO2 laser treatment.

Further study on the mechanism by which LLLT induces these changes suggests that LED causes changes in mitochondrial complexes I, II, III, IV, succinate dehydrogenase, and cytochrome C (part of complex IV) ultimately leading to increased nitric oxide (NO) and thus increased wound healing.

Given these past results, we now want to confirm response in human subjects.

Spitler and Berns: Comparison of laser and diode sources for acceleration of *in vitro* wound healing...



The primary endpoint of this study is time in days to complete healing of the treatment sites. Clinical grading shall be by blinded assessment, using recognized assessment techniques. A secondary outcome measure will be post-treatment pain on a 10-point scale.

Relevant references/articles to support the rationale for the research:

1. Chaves ME, Araújo AR, Piancastelli AC, Pinotti M. Effects of low-power light therapy on wound healing: LASER x LED. An Bras Dermatol. 2014;89(4):616-623. doi:10.1590/abd1806-4841.20142519
2. Spitler R, Berns MW. Comparison of laser and diode sources for acceleration of in vitro wound healing by low-level light therapy. J Biomed Opt. 2014;19(3):38001. doi:10.1117/1.JBO.19.3.038001
3. Whelan HT, Smits RL Jr, Buchman EV, et al. Effect of NASA light-emitting diode irradiation on wound healing. J Clin Laser Med Surg. 2001;19(6):305-314. doi:10.1089/104454701753342758
4. de Oliveira RA, Fernandes GA, Lima AC, Tajra Filho AD, de Barros Araújo R Jr, Nicolau RA. The effects of LED emissions on sternotomy incision repair after myocardial revascularization: a randomized double-blind study with follow-up. Lasers Med Sci. 2014;29(3):1195-1202. doi:10.1007/s10103-013-1503-2
5. Barolet D. Light-emitting diodes (LEDs) in dermatology. Semin Cutan Med Surg. 2008;27(4):227-238. doi:10.1016/j.sder.2008.08.003
6. Spitler R, Ho H, Norpetlian F, et al. Combination of low-level light therapy and nitrosyl-cobinamide accelerates wound healing. J Biomed Opt. 2015;20(5):051022. doi:10.1117/1.JBO.20.5.051022
7. Avci P, Gupta A, Sadasivam M, et al. Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring. Semin Cutan Med Surg. 2013;32(1):41-52.
8. Trelles MA, Allones I, Mayo E. Er:YAG laser ablation of plantar verrucae with red LED therapy-assisted healing. Photomed Laser Surg. 2006;24(4):494-498. doi:10.1089/pho.2006.24.494
9. Whelan HT, Smits RL Jr, Buchman EV, et al. Effect of NASA light-emitting diode irradiation on wound healing. J Clin Laser Med Surg. 2001;19(6):305-314. doi:10.1089/104454701753342758

Research Procedures

Methodology: Informed consent and screening prior to commencement of the study.

Day 0. Visit 1 (Baseline).
Baseline assessment of Fitzpatrick skin type, ethnicity, demographics and general medical history and status, concomitant medications.
Clinical digital photography of treatment site prior to laser. Subjects will be randomized to which arm denotes the test or control side.
Appropriate eye protection in the form of dedicated laser goggles will be provided to the patient and all other individuals in the treatment room. Ablative fractional laser treatment test area will be (5.5% density/700um) delivered to both inner biceps, close to the underarm to an approximately 3 x 3 cm square area. Subjects will evaluate pain and discomfort immediately after the treatment using an 11-point Visual analogue scale.
Clinical digital photography of test and control site post induction will be recorded. Immediately after photography the test area will be irradiated with the Biophotas Celluma for 30 minutes. The Celluma light panel will be disinfected prior to every use over open wound. The Control side will be untreated. The Celluma POD device will not be used over known cancer tumor or metastasis.
30 minutes after treatment with the Biophotas Celluma subjects will evaluate pain and discomfort for test and control sites using an 11-point Visual analogue scale.

Days 1-27 – treatment with Celluma
Test site will receive a 30-minute treatment of Celluma, 3 treatments per week for 4 weeks, with the first treatment being day 0 immediately after ablative fractional laser treatment test area. Day 1, 3, 6 subjects will record daily site symptoms including pain and discomfort and complete a treatment log in a patient diary.

Days 2*, 4*, 6*, 9, 13, 20, and 27 – Assessments
* Scheduled follow-up visits may be adjusted 1-2 days based on clinic or patient availability.
Clinical digital photography of test and control site will be recorded. Investigator assessment of treatment response using visual analogue scales to include Time to heal, Percentage healing, edema, erythema, swelling and crusting.
Subject assessment of treatment response using a grading of response.

Day 34 – Assessments Clinical digital photography of test and control site will be recorded. Investigator assessment of treatment response using visual analogue scales to include Time to heal, Percentage healing, edema, erythema, swelling and crusting.
Subject assessment of treatment response using a grading of response.

Day 55 – Final follow up
Clinical digital photography of test and control site will be recorded.
Investigator assessment of treatment response using visual analogue scales to include Time to heal, Percentage healing, edema, erythema, swelling, crusting, discoloration, and scarring
Subject assessment of treatment response using a grading of response.
If either or both skin wounds have not completely healed by this time, an additional follow-up visit will be scheduled at day 62 (one week after original final follow-up visit).

Study Narrative and Baseline Day 0
1. After completing informed consent and photography release forms subjects will be enrolled into the study.
2. Subjects will be randomized to which arm denotes the test or control side.
3. The clinical investigator will assess baseline characteristics including assessment of Fitzpatrick skin type, ethnicity, demographics and general medical history and status, and concomitant medications.
4. Subjects will be instructed on the use of the Biophotas Celluma LED device and observed switching the device ON/OFF, positioning the device and after treatment care of the device.
5. Clinical digital photography of treatment site will be recorded immediately prior to treatment
6. Ablative fractional laser treatment test area (5.5% density/700um) will be delivered to both inner biceps, close to the underarm an approximately 3 x 3 cm sq area.
7. Immediately after the test area administration, subjects will evaluate pain and discomfort using an 11- point Visual analogue scale.
8. Clinical digital photography of test and control site post induction will be recorded.
9. Immediately after photography the test wound will be irradiated with the Biophotas Celluma for 30 minutes. The Control side will be untreated. Subjects will be observed self-administrating the treatment.
10. 30 minutes after treatment with the Biophotas Celluma subjects will evaluate pain and discomfort for test and control sites using an 11-point Visual analogue scale.
11. Subjects will be instructed to treat the test site daily for 28 days and to complete their treatment log and diaries daily. Subjects will be given contact details for any adverse incidents or questions they may have.
12. Subjects will be given a date and time to return to the clinic for assessment.

Days 1-27
1. Subjects will complete their diaries and treatment log daily.
2. Daily, immediately before and 30 minutes after each Biophotas Celluma treatment the subject will record pain and discomfort.
3. From Day 2-27 subjects will record itching immediately before and 30 minutes after each Biophotas Celluma treatment.

Days 2*, 4*, 6*, 9, 13, 20 and 27 – Assessments
1. Subjects will return to the clinic on days 2, 4, 6, 9, 13, 20 and 27. * Scheduled follow-up visits may be adjusted 1-2 days based on clinic or patient availability.
2. The subject will be questioned generally to ascertain any changes in health or concomitant medication.
3. The subject's diary will be reviewed, and the subject will be questioned to ascertain any potential adverse incidents.
4. Clinical digital photography of test and control site will be recorded.
5. The Investigator will assess both test and control sites using visual analogue scales to include time to heal, percentage healing, edema, erythema, swelling and crusting.
6. Subject will assess treatment response using a grading of response.
7. The subject will be given a date and time to return to the clinic for the next assessment.

Day 34
1. The subject will be questioned generally to ascertain any changes in health or concomitant medication.
2. Clinical digital photography of test and control site will be recorded.
3. The Investigator will assess both test and control sites using visual analogue scales to include time to heal, percentage healing, edema, erythema, swelling and crusting.
4. Subject will assess treatment response using a grading of response.
5. The subject will be given a date and time to return to the clinic for the last assessment.

Day 55
1. The subject will be questioned generally to ascertain any changes in health or concomitant medication.
2. Clinical digital photography of test and control site will be recorded.
3. The Investigator will assess both test and control sites using visual analogue scales to include time to heal, percentage healing, edema, erythema, swelling and crusting.
4. Subject will assess treatment response using a grading of response. If there are no continuing adverse events and the patient has completed all diaries, they will be allowed to keep the Celluma device, and signed off the study.

Digital photographs will be taken of each ventral arm from antecubital fossa to axilla before laser application, directly after laser application, and at designated assessment visits in clinic. The photos will only include the ventral arm in the frame. If there are tattoos present in the area they will be covered using a black photography cloth or blue medical drape.
Subject will be asked to give consent prior to participating in the study by signing an informed consent form and photography release form.
Publications and/or presentations that result from this study will not include subject identifiable information.

Time in study 56 days from Baseline visit (day 0)

Study Design

This is a single-center, randomized single-control study. One arm will serve as control and will not receive Celluma LED device treatment. The laterality of the control and test arms will be randomized.

Methods of evaluation
1. Fitzpatrick skin typing at baseline including ethnicity
2. General medical history and concomitant medications
3. Standardized clinical photography
4. Investigator assessment of treatment response using applicable scales to include:
   i. Time to heal
   ii. Percentage healing
   iii. Edema iv. Erythema
   iv. Crusting
   v. Discoloration
   vi. Scarring
5. Subject assessment of pain, discomfort, and itching post treatment
6. Patient diaries
7. Adverse event recording

Methods of assessment
Authors note:
Scales used to describe healing in leg ulcers and general or surgical wounds, apart from the Leg Ulcer Measurement Tool LUMT, lack comprehensive and quality evaluation with respect to validity, reliability, and sensitivity. The author has reviewed the Food and Drug Administration's guidelines on wound healing and has identified the following areas that constitute important measurement metrics in wound healing.

Clinical assessment
Clinical photography
Digital clinical photography shall be taken at baseline, immediately post treatment and at each assessment point.

Healing assessment
Clinical assessment shall be performed by independent and blinded assessors, based on visual examination and clinical photography of the test and control areas at baseline (before the first session), and at each assessment point.

Healing shall be graded at each assessment on a 5-point visual analogue scale as follows:

i.	Excellent:	81% - 100% healing
ii.	Very Good:	61% - 80% healing
iii.	Good:	41% - 60% healing
iv.	Fair:	21% - 40% healing
v.	Little or no change:	0% - 20% healing

Erythema
Clinical assessment shall be performed by independent blinded assessors, based on visual examination and clinical photography of the test and control areas at baseline (before the first session), and at each assessment point.

Erythema shall be graded at each assessment on an 11-point scale as follows:
i. Little to No Erythema
- 0
- 1
ii. Mild Erythema
- 2
- 3
- 4
iii. Moderate Erythema
- 5
- 6
- 7
iv. Severe Erythema
- 8
- 9
- 10

Swelling
Clinical assessment shall be performed by independent and blinded assessors, based on visual examination and clinical photography of the test and control areas at baseline (before the first session), and at each assessment point.

Swelling shall be graded at each assessment on a 11-point scale as follows:
i. No Swelling evident
- 0
- 1
ii. Mild Swelling
- 2
- 3
- 4
iii. Moderate Swelling

- 5
- 6
- 7

iv. Severe Swelling

- 8
- 9
- 10

Crusting

Clinical assessment shall be performed by independent and blinded assessors, based on visual examination and clinical photography of the test and control areas at baseline (before the first session), and at each assessment point.

Crusting shall be graded at each assessment on a 11-point visual analogue scale as follows:

i. No crusting evident

- 0
- 1

ii. Mild Crusting

- 2
- 3
- 4

iii. Moderate Crusting

- 5
- 6
- 7

iv. Severe Crusting

- 8
- 9
- 10

Subject assessment

Pain

Pain shall be assessed using an 11-point Visual Analogue Scale (VAS) (0-10-point scale). The Scale will be used at the following timepoints.

i. Immediately after the test area performance
ii. 30 minutes after the first treatment session of Biophotas Celluma.
iii. Daily, immediately before and 30 minutes after each Biophotas Celluma treatment.

Discomfort

Discomfort shall be assessed using an 11-point Visual Analogue Scale (VAS) (0-10-point scale). The Scale will be used at the following timepoints.

i. Immediately after the test area performance
ii. 30 minutes after the first treatment session of Biophotas Celluma
iii. Daily, immediately before and 30 minutes after each Biophotas Celluma treatment

Itching

Itching shall be assessed using an 11-point Visual Analogue Scale (VAS) (0-10-point scale). The Scale will be used at the following timepoints.

Daily, immediately before and 30 minutes after each Biophotas Celluma treatment.

STATISTICAL ANALYSIS PLAN

The primary endpoint measured in the study is length of days to reach either a 0 or 1 on the 11-point scale for erythema, swelling, and crusting, as well as 80-100% overall healing on the treatment arm compared to the control arm. Comparisons of pain, discomfort, and itch ratings will also be made between treatment and control arms by calculating the average scores.

Times to full healing and times to resolution of all side effects will be described by frequency distributions together with mean values and associated 95% confidence limits.

A comparable study of LED therapy involving 10 patients gave mean (SD) treated healing time of 13.5 (1.1) days and mean (SD) untreated healing time of 26.8 (1.6) days. Mean (SD) of relative reduction in healing time was 50% (2.2%) and the lowest relative reduction was 48%. Assuming similar results for the proposed study, the study size of 25 would virtually guarantee statistically significant reductions in healing time. Based on 95% confidence intervals, the mean treated healing time, mean untreated healing time and mean relative reduction in healing time will be estimated with precisions of 3.1%, 2.3% and 1.7%.