AMERICAN SOCIETY FOR LASER MEDICINE AND SURGERY

BASIC SCIENCE AND TRANSLATIONAL RESEARCH: *CLINICAL THERAPEUTICS*

A DOSE-RESPONSE STUDY OF A NOVEL NON-THERMAL METHOD OF SELECTIVELY MODIFYING CELLULAR STRUCTURES IN SKIN WITH LOW ENERGY NANOSECOND ELECTRICAL STIMULATION

David Kaufman, Michelle Martinez, Brian D. Zelickson, Edward A. Ebbers, Richard Nuccitelli, Lauren Jauregui, Darius Mehregan

Kaufman Plastic Surgery, Inc., Folsom, CA; Zel Skin and Laser Specialists, Edina, MN; Pulse Biosciences, Inc., Hayward, CA; Wayne State University School of Medicine, Detroit, MI

Background: A novel modality for modifying cellular structures of the epidermis and dermis through the application of a nano-pulse stimulation (NPS) electrical energy device to normal skin was evaluated in a first-in-human clinical study of dose-response effects.

Study Design/Materials and Method: Five patients with healthy tissue planned for abdominoplasty excision were enrolled in a 60-day study of effects of a novel method for modifying tissue using low energy, high voltage NPS. A total of 30 squares of 25 mm 2 or less within the planned excision area were treated at 60 days, 30 days, 15 days, 5 days and 1 day prior to surgery, using 6 progressively higher NPS energy levels. Photographs were taken of each treated area. Five different staining methods were used to assess tissue changes.

Results: The majority of test sites exhibited delayed epidermal loss followed by re-epithelialization by Day 15 and a normal course of healing. Histologic analysis identified a nucleolysis effect on epidermal cells. Minimal effects were seen on melanocytes, elastic fibers and collagen when compared with controls at day 60 in the majority of evaluated tissue samples, with notable exceptions of 2 tested areas treated at the higher energy levels that showed signs of dermal damage.

Conclusion: The selective effect of NPS on cellular structures in the epidermal and dermal layers suggests a non-thermal mechanism for targeting cellular structures that does not affect the viability of dermal tissue within a range of energy levels. These histology results indicate that the lowest effective energy level was not established in this study. The specificity of effects and a favorable healing response lends itself to cellular targets in the epidermal or dermal layers of the skin, including treatment of benign and non-benign lesions. NPS skin treatments provide a promising, non-thermal method for treating skin conditions and removing epidermal lesions.

EXTRACELLULAR VESICLE EXOCYTOSIS ARE UPREGULATED IN PORT WINE STAIN BLOOD VESSELS

Rong Yin, Wenbin Tan, Shawn Rice, Xin Liu, J. Stuart Nelson

Beckman Laser Institute and Medical Clinic, University of California, Irvine, CA; Penn State Cancer Institute, Hershey, PA

Background: Port wine stain (PWS) is characterized by a progressive dilatation of immature venule-like vasculatures which result from differentiation-impaired endothelial cells. In this study, we aimed to identify the major biological pathways accounting for the pathogenesis of PWS.

Study Design/Materials and Method: Sequential windowed acquisition of all theoretical fragment ion mass spectra (SWATH-MS) was used to identify differentially expressed proteins in PWS lesions, followed-up by confirmative studies with immunohistochemistry, immunoblot and Transmission Electron Microscopy (TEM).

Results: One hundred seven out of 299 identified proteins showed differential expressions in PWS lesions as compared to normal skin, mainly involving the functions of biosynthesis, membrane trafficking, cytoskeleton and cell adhesion/migration. The confirmative studies showed that expressions of cell adhesion/migration/exocytosis related proteins such as VAT1, IQGAP1, HSC70, clathrin, perlecan, spectrin α1 and GDIR1 were significantly increased in PWS blood vessels; while collagen subtypes 6A1 and 6A3 were decreased in PWS skin. Furthermore, TEM studies showed there is a significant upregulation of extracellular vesicle exocytosis from PWS blood vessels as compared to control.

Conclusion: Our results showed that (1) PWS blood vessels have increased expressions of cell adhesion/migration/exocytosis related molecules; (2) extracellular vesicles show increased exocytosis from PWS blood vessels, which carry and mediate critical intercellular signaling in the pathogenesis of PWS; and (3) dysregulation of collagen 6 contributes to collagenous alterations in PWS skin.

MULTIMODAL MONITORING OF HYPERTHERMIC LASER LIPOLYSIS WITH 1064 nm Nd:YAG LASER IN HUMAN SUBJECTS

Matija Milanic, Blaz Tasic, Jost Stergar, Matjaz Lukac

University of Ljubljana, Ljubljana, Slovenia; Fotona d.o.o., Ljubljana, Slovenia; Jozef Stefan Institute, Ljubljana, Slovenia

Background: The aim of this study is to experimentally monitor perfusion and temperature during the minimally invasive hyperthermic lipolysis (HTLL) in human subjects in order to improve the understanding and the treatment parameters of the procedure.

Study Design/Materials and Method: Hyperthermic 1064 nm Nd:YAG laser lipolysis was performed in one healthy volunteer with skin type II. The irradiated area was approx. $10 \times 10\,cm^2$ (Fotona) the irradiance was $1.2\,W/cm^2$. Different irradiation scenarios were considered, including natural convection only cooling, forced air cooling and sequential irradiation involving multiple irradiation ant thermal relaxation phases. The diffuse reflectance spectrum (DRS) of the irradiated skin in the

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400–1000 nm spectral range was measured before and after the irradiation. The skin surface was additionally continuously monitored with an infrared camera and an RGB-camera with 1,064 nm cut-off filter.
Results: From the RGB-video blood-volume-fraction (bvf) evolution was determined by calculating the custom bvf-indices corresponding to the skin bvf. The DRS served as a calibration standard for the RGB-bvf. The RGB-bvf was compared to the measured temporal temperature evolution and a good agreement between the elevated RGB-bvf and reduced temperature rise rate was observed. The determined RGB-bvf and temperature evolution served as an input into a 1D model of the treatment including heat transport and blood perfusion. By solving the inverse problem the model provided information about temperature depth evolution and allowed for estimation of laser-induced tissue damage by calculating corresponding Arrhenius integral.
Conclusion: The study demonstrated that the combined thermal and multispectral imaging of HTLL provided valuable information about both temperature and blood perfusion evolution. This information could be further used to predict temperature depth evolution and tissue damage during the procedure, thus improving treatment outcome and safety.

NON-INVASIVE ASSESSMENT OF CHANGES IN HUMAN SKIN UPON FRACTIONAL LASER REMODELING

Nina Verdel, Ana Marin, Matjaz Lukac, Boris Majaron
Jozef Stefan Institute, Ljubljana, Slovenia; Faculty of Mathematics and Physics, Ljubljana, Slovenia

Background: Our group has recently introduced an innovative methodology for non-invasive assessment of structure and composition of human skin *in vivo*. The technique combines diffuse reflectance spectroscopy (DRS), pulsed photothermal radiometry (PPTR), and analysis based on a numerical model of light transport in human skin. In this study we test its ability to assess the changes in scattering properties of skin upon fractional laser remodeling.
Study Design/Materials and Method: The study involved 7 healthy volunteers with Fitzpatrick skin types I–II (age 24–63). Two test sites ($9 \times 9 mm^2$) on the inner forearm of each volunteer were treated with two types of fractional skin rejuvenation lasers, a sub-millisecond Er:YAG (equipped with a 9×9 pixel handpiece FS01), and a nanosecond Nd:YAG laser (with 9×9 pixel handpiece FS20A), both from Fotona, Slovenia. A third site served as a negative control. DRS in visible spectral range (400–650 nm) were acquired using an integrating sphere (ISP-REF by Ocean Optics). PPTR measurements involved 1 ms laser pulses at 532 nm and recording of transient change in mid-infrared emission with a fast infrared camera (FLIR, SC7500) at 1000 frames per second. Measurements were performed before, one month after, and three months after treatment, and the skin properties were assessed by objective fitting of experimental data with predictions of a dedicated numerical model.
Results: Our preliminary results indicate an increase of the reduced scattering coefficient of skin and increase of the characteristic power in its spectral dependence after fractional skin rejuvenation treatment, in agreement with anticipated remodeling of dermal collagen. The study will be completed and this abstract updated well before the Conference.
Conclusion: Our study demonstrates that the described methodology allows non-invasive assessment of dermal collagen remodeling upon laser skin rejuvenation. This enables non-invasive monitoring of skin response to skin rejuvenation and optimization of treatment parameters.

BASIC SCIENCE AND TRANSLATIONAL RESEARCH: *IMAGING AND SENSING*

ABLATIVE FRACTIONAL LASER ENABLES TOPICAL DELIVERY OF VISMODEGIB *IN VITRO*

Uffe Høgh Olesen, Gael Clergeaud Veiga, Catharina M. Lerche, Thomas Lars Andresen, Merete Haedersdal
Bispebjerg Hospital, Copenhagen, Denmark; Technical University of Copenhagen, Lyngby, Denmark

Background: Hedgehog inhibitors such as vismodegib are targeted drugs for the treatment of basal cell carcinomas. Their use is significantly limited by frequent and severe systemic side effects due to their oral administration. We aim to develop a topical treatment with vismodegib using ablative fractional laser (AFXL) and emulsion vehicle with the perspective of a gentler and more efficient treatment of basal cell carcinomas.
Study Design/Materials and Method: *In vitro* pig skin was treated with a fractional 10,600 nm CO_2 laser at 0 or 80 mJ/microbeam and exposed to vismodegib in Franz-diffusion cells for 0.5, 4 and 24 hours (drug: $n = 48$ samples, untreated: $n = 6$ samples). Vismodegib (9.0 mM) was dissolved in either emulsion vehicle consisting of soy bean oil and Tween80 or ethanol as control. Vismodegib biodistribution was studied at increasing depths from 0 μm to 1800 μm (incremental steps of 300 μm) by liquid chromatography mass spectrometry.
Results: Combination of AFXL and emulsion vehicle enables delivery of vismodegib into the skin. Emulsion vehicle yielded higher vismodegib skin concentrations compared to ethanol control both with laser (0–900 μm, $p = 0.002$) and without (0–600 μm, $p = 0.002$–0.015). In superficial skin layers, 0–300 μm, combining laser and emulsion for 24 hours resulted in a skin concentration of 568 μM, 6.3 fold higher than laser and ethanol ($p = 0.002$). For emulsion, the uptake of vismodegib increases significantly over time (4h–24h, $p = 0.002$–0.004). Compared to unexposed skin, AFXL pretreatment significantly increases skin concentrations of vismodegib (emulsion: 300–1800 μm, $p = 0.004$–0.048; ethanol: 600–1800 μm, $p = 0.002$–0.015). In deeper skin layers, 900–1200 μm, 99 μM was obtained combining laser and emulsion for 24 hours, corresponding to a 9.9 fold increase compared to emulsion alone ($p = 0.004$).
Conclusion: AFXL enables topical delivery of vismodegib into deeper skin compartments and in a time dependent manner resulting in high therapeutic skin concentrations.

ASSESSMENT OF BACTERIA GROWTH UNDER TRANSPARENT NANOCRYSTALLINE YTTRIA-STABILIZED-ZIRCONIA CRANIAL IMPLANT USING LASER SPECKLE IMAGING

Nami Davoodzadeh, Natanael Cuando, Amir Hessam Aminfar, Mildred Cano, Guillermo Aguilar
University of California – Riverside, Riverside, CA; National Autonomous University of Mexico, Mexico City, Mexico

Background: Laser-based diagnostics and therapeutics show promise for many neurological disorders. However, the poor transparency of cranial bone limits the spatial resolution and interaction depth that can be achieved. We addressed this limitation previously, by introducing a novel cranial prosthesis made of a transparent nanocrystalline yttria-stabilized-zirconia

(nc-YSZ) which we call Window to the Brain (WttB) implant. WttB aims to enhance the diagnosis and treatment of neurological diseases by providing chronic optical access to the brain. By using optical coherence tomography and laser speckle imaging, we have demonstrated the initial feasibility of nc-YSZ implants for cortical imaging in an acute murine model. However, bacterial adhesion to the cranial implant is the leading factor for biofilm formation (fouling), infection, and implant failure. Escherichia coli (E. coli), in particular, represents the most common isolate in gram-negative bacillary meningitis after cranial surgery or trauma.
Study Design/Materials and Method: The transparency of our WttB implant may provide a unique opportunity for non-invasive monitoring of bacterial infection under the implant using laser speckle imaging (LSI). In this present work, phase and amplitude distribution changes of the light in time caused by the bacteria growth in an *in vitro* model is studied using LSI.
Results: Time-varying speckled structures called biospeckle patterns was observed as a result of bacteria growth. Moreover, our results suggest that the dynamics of biospeckles are related to the bacterial growth rate.
Conclusion: The results of this work suggest LSI as a non-invasive tool for early detection of bacterial infection and assessment the antibacterial treatments.

ASSOCIATION OF CHANGES IN TISSUE OPTICAL PROPERTIES WITH THERMAL TISSUE DAMAGE

Vivek Krishna Nagarajan, Bing Yu
Marquette University, Milwaukee, WI

Background: Complete thermocoagulation of tumours is vital for minimizing the risk of local tumor recurrence. Physical quantities that are indicative of tissue damage status are needed for continuous monitoring during thermocoagulative procedures. In our previous studies, we found that changes in wavelength-averaged tissue absorption coefficient (μa) and reduced scattering coefficient ($\mu s'$) of porcine liver tissue during thermocoagulation, measured with diffuse reflectance spectroscopy (DRS) in the visible range (435–630 nm), followed a sigmoidal growth curve. We hypothesize that increases in tissue μa and $\mu s'$ during thermal ablation are correlated with thermal damage.
Study Design/Materials and Method: An integrated fiber-optic probe was used to continuously record the local tissue temperature and diffuse reflectance spectra during liver tissue heating. Fresh porcine liver tissues samples (10 in each group) were heated to 37°C (group 1), 55°C (group 2), 65°C (group 3) & 75°C (group 4), respectively. The tissue $\mu a(\lambda)$ and $\mu s'(\lambda)$ are estimated from the measured diffuse reflectance spectrum using an inverse Monte-Carlo model in real-time. The continuously measured μa and $\mu s'$ are statistically compared with the histological-assessed true tissue damage for all samples.
Results: The mean μa of groups 1&2 were not significantly different from each other. The mean μa of groups 3&4 were significantly different ($p < 0.05$) from each other and from the other groups. Similarly, the mean $\mu s'$ of groups 1&2 were significantly different from each other and from groups 3&4. The mean $\mu s'$ of groups 3&4 were not significantly different from each other. The histological-assessed damage scores for groups 3&4 were significantly different from each other as well as other groups. However, the damage scores were not significantly different between groups 1&2.
Conclusion: Both μa and $\mu s'$ increased during liver tissue heating and were correlated with true tissue damage. The data suggests that optical monitoring of thermocoagulation procedures could potentially be used to assess tissue damage in real-time.

CHANNEL-COMPRESSED RAMAN SPECTROSCOPY FOR FAST ACQUISITION AND DETECTION OF TUMOUR MARGINS

Xu Feng, Austin J. Moy, Hieu Nguyen, Matthew Fox, Katherine Sebastian, Jason Reichenberg, James W. Tunnell
University of Texas at Austin, Austin, TX

Background: While Raman microspectroscopy (RMS) has shown great promise as a label-free method to guide tumour resection procedures, it could benefit from further improvements in acquisition speed. Typical RMS systems for spontaneous Raman acquisition of tissues use CCD detectors with 1024 spectral channels (pixels). Here, we investigate the strategy of channel compression for reducing the number of spectral channels to a minimum without loss of accuracy.
Study Design/Materials and Method: We used a Raman data set derived from basal cell carcinoma (42 spectra, 15 patients) and normal structures (116 spectra, 25 patients) collected with 1024 spectral channels. Using our previously established biophysical model, we extracted the biochemical composition of collagen, elastin, triolein, nucleus, keratin, ceramide, and built discrimination models with corresponding receiver operator characteristics curves (ROCs). We then used software binning to compress the number of channels, and determined the diagnostic performance of the biophysical model as a function of channel number.
Results: Simulation results show that for channel numbers greater than or equal to 32, area under the ROC curve was similar to 1024 channels. When channels were compressed from 32 to 16, area under the ROC curve decreased 7% and root-mean-square-error (RMSE) of biophysical features increased substantially. The diagnostic power rapidly decreased with channel numbers less than 8.
Conclusion: To our knowledge, uniformly spaced sequential channel compression has not been employed with spontaneous RMS for cancer detection. Our results show that the minimum number of channels to maintain the original diagnostic power is 32, a reduction of more than an order of magnitude. This opens up the possibility for significantly increasing the speed of RMS without using complex compression strategies, moving it closer to a tool for intraoperative guidance of tumour margin detection. Future implementations could involve hardware binning to increase signal-to-noise ratio and replacing the spectroscopic camera with multi-channel detector arrays such as PMTs or avalanche photodiodes.

COMPARING AN IMAGING-BASED VERSUS SALIVA-BASED APPROACH TO DETERMINING ORAL CANCER RISK

Delaney Islip, Afarin Golabgir Anbarani, Kevin Lai, Betul Karaca, Pelin Guneri, Cherie Wink, Petra E. Wilder-Smith
University of California, Irvine, CA; ECE University, Izmir, Turkey

Background: Objective of this study was to investigate and compare the ability of imaging-based biomarkers versus salivary transcriptomic biomarkers to identify oral cancer risk in oral red and white lesions. Long-term goal is to develop a non-invasive means of identifying and monitoring oral cancer risk in potentially premalignant lesions of the oral mucosa. Current techniques that rely on sequential

surgical biopsy suffer from poor compliance, sampling error, and the inability to identify appropriate sampling sites and timepoints.
Study Design/Materials and Method: This study was performed in full compliance with UCI IRB 2002–2805. In patients with oral leukoplakia and/or erythroplakia, lesions were imaged with Optical Coherence Tomography techniques (OCT) and photographed at 0, 1 and 3 months. Additionally, stimulated and unstimulated saliva was collected at these time-points using standard techniques. q-PCR was performed to validate 4 mRNA's. Additionally, isolated RNA was reverse transcribed into cDNA using RNA-directed DNA polymerase. The resulting cDNA was used for PCR amplification for each biomarker. A blinded pre-standardized examiner scored OCT images on a semi-quantitative scale of 0–3 for level of pathology. Data were compared with the existing gold standard: histopathology by a standard external oral pathology laboratory. All subjects were monitored and treated according to the existing standard of care.
Results: Eight subjects with leuko- and or erythroplakia were enrolled, of whom 5 completed this pilot study. Over the study duration, the lesions appeared clinically unaltered. However, changes to mRNA markers IL1B and IL8 were observed over time. In the 1 patient who progressed to oral squamous cell carcinoma, significantly raised levels of miR181c and miR181b were identified. Using a previously developed segmentation approach to the OCT data, imaging-based diagnoses were in agreement with histopathological diagnosis in 13/15 evaluations (kappa value: 5 subjects, 3 timepoints each).
Conclusion: OCT-based diagnosis showed good agreement with histopathology, but little predictive value. Salivary biomarkers showed potential as indicators of Oral Cancer Risk. This research was supported by the National Institutes of Health under grants No. 1R03EB014852, UH2 EB022623, P41EB015890 and UL1 TR000153, as well as the Beckman Foundation.

DEPTH RESOLVED QUANTITATIVE PROFILING OF STRATUM CORNEUM LIPIDS AND WATER CONTENT USING SHORT-WAVE INFRARED SPECTROSCOPY AND CONFOCAL RAMAN SPECTROSCOPY

Anna Ezerskaia, Silvania Pereira, Paul Urbach, Babu Varghese
Delft University of Technology, Delft, South Holland, Netherlands; Philips Research, Eindhoven, Nord Brabant, Netherlands

Background: The permeability barrier function of the skin is provided by the stratum corneum (SC), the outermost layer of the skin. The intercellular lipids and the degree of hydration of the SC are the crucial determinants required for a competent skin barrier. Even though the SC is a non-uniform, inhomogeneous membrane, the question "whether the lipid and water composition is distributed uniformly across the SC thickness?" is not well addressed. Previous studies report the analysis of lipids and water based on the extracts of the entire SC and quantitative information on how the lipid and water composition changes with the depth in the SC is very limited.
Study Design/Materials and Method: The relative amount of water and lipid content in the SC with respect to the baseline was measured on the forehead (T-zone) of a clinically healthy female volunteer (26 years) after each tape stripping using our short wave infrared spectroscopic experimental set-up and the results are compared with Corneometer, Sebumeter, and Transepidermal water loss (TEWL) instrument. The infrared spectroscopic setup that we have developed utilizes differential detection with three wavelengths 1720 ± 4 nm, 1750 ± 5 nm, 1770 ± 20 nm corresponding to the lipid vibrational bands that lay "in between" the prominent water absorption bands. We performed additional independent Raman measurements using a confocal Raman microspectrometer (RiverD) as a reference method for comparison. We estimated the lipid and water content from the Raman spectra based on lipid to protein and water to protein ratio respectively (lipid: 2790–2910 cm-1; protein: 2910–2966 cm-1; water: 3350–3550 cm-1).
Results: As the number of tape-strips increases, the level of SC hydration also increases, which indicate progressively increasing H-bonding further and further into the SC reflecting, increasing availability of water. We observe a steeper change in water content for the first few tapes and thereafter, there is a much less dramatic decline with tape-strip number. We measure decrease in SC lipid content as a function of depth using short-wave infrared and Raman spectroscopic methods. This is consistent with previously reported microscopic histology, which showed dense corneocytes surrounded by relatively large amounts of lipid near the skin surface and larger corneocytes with smaller intercellular spaces deeper in the membrane. Sebumeter is sensitive only to non-bound sebaceous surface lipids. The spectroscopic method is sensitive to both bound and non-bound lipids and therefore makes it possible to measure the changes for lipids present in SC during tape stripping.
Conclusion: We quantify the depth resolved changes in SC lipids and hydration using short wave infrared spectroscopic set-up combined with tape stripping and compare the results with conventional biophysical devices such as Corneometer, Sebumeter Aqua Flux TEWL and confocal Raman spectrometer. It appears that the SC barrier in terms of its intracellular lipid and the degree of hydration is not uniform across its entire thickness: the outer few layers appear to be less hydrated and contains increased amount of intercellular lipids than the deeper layers of the membrane. We anticipate that short wave infrared spectroscopic technique combined with tape stripping can be used as a non-invasive, easy-to-apply low cost method for analyzing the SC components and thereby provide much more quantitative and more reliable skin barrier function information in contrast to conventionally employed biophysical methods.

DIFFUSE REFLECTANCE SPECTROSCOPY OF BREAST TUMOURS TO DIFFERENTIATE BETWEEN INDOLENT AND AGGRESSIVE DISEASE

Joel Rodriguez Troncoso, Narasimhan Rajaram
University of Arkansas, Fayetteville, Arkansas

Background: Metastatic spread is the primary cause of death among breast cancer patients. Clinically, doctors rely on anatomical markers, such as tumour stage and lymph node status for decisions related to neoadjuvant chemotherapy. However, for early-stage breast cancer patients with tumours that are not likely to metastasize, the side effects of chemotherapy can outweigh the benefits. The ability to differentiate between indolent and aggressive breast cancers based on the functional information from the primary tumour could significantly decrease the numbers of patients receiving unnecessary chemotherapy. In this study, we used diffuse reflectance spectroscopy (DRS) to assess functional changes within tumour xenografts derived from 3 breast cancer cell lines of varying metastatic potential.

Study Design/Materials and Method: The 4T1 (highly metastatic) and 67NR (non-metastatic) murine mammary adenocarcinoma cells were injected into the flanks of 10 mice each to grow xenografts. In addition, we used CRISPR/Cas-9 to delete TWIST, which promotes tumour metastasis, and generate an indolent version of the 4T1 (4T1-TKO) that was also grown as xenografts. A fiber probe-based optical spectrometer was used to acquire DRS spectra from tumours using a source-detector separation of 2.25 mm. DRS spectra were acquired at tumour volumes corresponding to 50, 100, 150 and 200 mm^3. Using a lookup table-based inverse model, we determined scattering, total concentration of hemoglobin (cHb), and the oxygen saturation (SO_2). *In vitro* oxygen consumption was determined using Seahorse metabolic flux analyzer.
Results: We observed significant differences in cHb and sO_2 between the metastatic 4T1 tumours and the non-metastatic 4T1-TKO and 67NR tumour populations. In addition, the direction of change in optical properties as a function of tumour volume was different for the three groups.
Conclusion: Our preliminary results indicate functional differences between indolent and aggressive tumours that can be further investigated in human cell line and patient-derived tumours.

FIRST REPORT OF OPTICAL PROPERTIES OF HUMAN NEUROFIBROMA TUMOURS

Yidan Sun, William A. Farinelli, Ying Wang, Curtis L. Cetrulo, Scott R. Plotkin, R. Rox Anderson, Fernanda H. Sakamoto
Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA

Background: Neurofibromatosis (NF) is the most common genetic neurological disorder of mankind. Cutaneous neurofibromas progress from small invisible lesions in children, to large itchy and disfiguring tumours in adults. There is no known cure for NF. Surgical resection or destruction are the only treatments used for skin lesions. Ideally, a tumour-selective optical treatment could be devised, to eliminate tumours before they become a clinical burden. We investigated the optical properties of human NF tumours.
Study Design/Materials and Method: The optical properties of 6 freshly excised human neurofibromas from 4 subjects were studied in an IRB-approved protocol. Epidermis was removed by suction blistering, then 1 mm thick fresh vertical sections were placed between two microscope slides. An integrating sphere spectrophotometer was used to measure the total reflectance and transmittance spectra of the NF lesions. Normal human dermis was used as a comparative control.
Results: The diagnosis of cutaneous NF was confirmed histologically for all study lesions. Optical data were consistent among the samples. Absorption maxima were observed at ~410,~540,~580,~970,~1185,~1450 and ~1790 nm. The visible bands are those of oxyhemoglobin, and the infrared bands are due to lipid and water. Similar spectra were obtained from normal dermis samples.
Conclusion: We report here, the first measurement of optical properties of human neurofibromas. No chromophore unique to NF tumours was identified. Optical scattering was apparently greater in dermis than NF tumours. Although no distinct chromophore was found in NF tumours, the potential exists that lasers targeting hemoglobin, water, or lipid could *in vivo* provide selective photothermal effects. This pilot study was limited to *in vitro* tissue, and a small number of tumour samples.

HANDHELD, WAVEGUIDE-MEDIATED, PHOTOACOUSTIC COMPUTED TOMOGRAPHY FOR BURN WOUND DEPTH ANALYSIS

Mason W. Schellenberg, Paul J. Whiteside, Amanda Sain, Heather K. Hunt
University of Missouri, Columbia, MO

Background: Approximately 500,000 patients are hospitalized for burn wounds in the United States annually. For these patients, diagnoses are performed via qualitative, clinical assessment, which is accurate in only 65–70% of cases. Misdiagnoses can lead to longer patient recovery times, reduced patient outcomes, and higher costs. Therefore, the ability to quantify burn metrics, such as wound depth, non-invasively could greatly improve the accuracy of clinical assessments and lead to better patient outcomes. To do this, imaging techniques, such as Photoacoustic computed tomography (PACT), could be employed. PACT is an imaging modality that has been used for imaging structures within the skin, and has been used in several preclinical trials to image tumours. PACT can also monitor blood flow and oxygenation, making it uniquely situated to monitor burn wounds by identifying burn wound zones.
Study Design/Materials and Method: However, PACT has several limitations, among which are the standard, opaque, ultrasound transducers used for signal acquisition. These transducers make it difficult to deliver light to tissue directly under the transducer. To overcome this, and to simultaneously deliver high quality images, we have developed and will demonstrate a handheld, PACT imaging system that uses an optical waveguide to deliver light to tissue directly underneath a transducer.
Results: Tissue phantoms made to mimic the acoustic and optical properties of dermis tissue are used to characterize the system; maximum imaging depth and image resolution, as well as the resulting effective imaging volume, will be explored in the presentation. These results are then compared to the capabilities of existing PACT systems to evaluate the efficacy of the new modality and holistic imaging system.
Conclusion: In the future, this system could be used to quantitatively measure burn wound depth, making it easier to correctly diagnose burns, and improve patient outcomes.

IMAGING AND CHARACTERIZING PHYSIOLOGY AND MORPHOLOGY OF MICROVASCULATURE IN SKIN USING OPTICAL COHERENCE TOMOGRAPHY

Malte J. Casper, Michael Evers, Garuna Kositratna, Gereon Huettmann, Dieter Manstein
Massachusetts General Hospital, Boston, MA; Universitaet zu Luebeck, Luebeck, Schleswig-Holstein, Germany

Background: Over the last two decades optical coherence tomography (OCT) has emerged to a valuable diagnostic tool for physicians of a variety of disciplines. Hereby, OCT is not limited to three-dimensional, high-resolution imaging of the morphology, but also functional information can be displayed, such as blood flow in the microvasculature of the skin. These angiographic images unveil the perfusion of the skin, which changes dynamically for thermoregulation or due to pressure. Long-term changes in the capillary architecture could be symptomatic for aging, inflammatory processes, disease development, wound healing. Here, we demonstrate how both, physiology and anatomy of the capillary network in human skin can be imaged and compared to derive valuable clinical insights.

Study Design/Materials and Method: Angiographic images were obtained with a commercially available OCT imaging device before and immediately after pressure was applied to the skin. While the baseline image displays the normal amount of perfused capillaries, the image after releasing the pressure shows the network of anatomically existing and perfusable capillaries. In these images, the capillary network was automatically detected and segmented using an optimized image processing pipeline. This enabled us to derive quantitative metrics of the vascular architecture such as vessel density, curvature and network complexity. These metrics are used to characterize the imaged capillary networks and allowed to compare among different imaging situations.
Results: At the baseline measurement, fewer capillaries were perfused and were of smaller diameter than after pressure was applied and released.
Conclusion: With this work, OCT angiography is extended with state-of-the-art methods of computer vision to a valuable diagnostic tool for characterization of vascular diversity. The disparity between actual and potential perfusion might differ between patients of different age and exposition and provide meaningful insights to the viability of skin perfusion.

MORPHOLOGICAL DIFFERENCES OF MILD TO MODERATE ACNE VULGARIS VISUALIZED BY REFLECTANCE CONFOCAL MICROSCOPY AND OPTICAL COHERENCE TOMOGRAPHY

Christine Fuchs, Amanda J.B. Andersen, Peter A. Philipsen, Mette Mogensen, Merete Haedersdal
Bispebjerg University Hospital, Copenhagen, Denmark

Background: Reflectance confocal microscopy (RCM) and optical coherence tomography (OCT) are *in vivo* imaging technologies that when combined provide an ideal non-invasive method to characterize acne lesions. RCM has spatial resolution to detect cellular morphology and OCT visualizes acne lesions in lower dermis with lower resolution. We aim to describe morphology of acne lesions by OCT and RCM, to compare hair follicles in normal skin to hair follicles in acne skin and to assess if RCM features of hair follicles are associated with acne severity.
Study Design/Materials and Method: Acne patients ($n = 14$) and healthy participants ($n = 7$) were included for RCM and OCT imaging. Open comedones, closed comedones, papules, and/or pustules on the face were imaged in patients; cheek and preauricular areas were imaged in healthy participants. Hair follicle morphology was described in RCM and OCT images and diameter evaluated by RCM.
Results: We investigated 473 hair follicles and 61 acne lesions. OCT differentiated open from closed comedones, identified papules and pustules, and assessed epidermal thickness. RCM visualized superficial characteristics of comedones, papules, and pustules. RCM of hair follicles demonstrated differences between healthy skin and acne. Higher acne grade was associated with more inflammation in apparently normal epidermis and dermis ($p = 0.00006$). Keratinous debris inside hair follicles was more frequent with increasing acne severity ($p = 0.006$). A hyperreflective ring around hair follicles was observed more frequently in acne patients ($p = 0.038$) and hair follicle diameter appeared larger ($p = 0.000003$).
Conclusion: Combined RCM and OCT imaging provided high-resolution *in vivo* morphological characteristics of specific acne lesions and hair follicles in patients with different acne severity degrees. Future clinical acne trials may benefit from implementing combined RCM and OCT imaging to characterize skin micromorphology and thus, provide important information on the mechanism of action for different treatment modalities.

MULTIPHOTON AUTOFLUORESCENCE MICROSCOPIC MEASURES OF ATYPIA IN ORAL AND OROPHARYNGEAL NEOPLASIA

Paula Villarreal, Rahul Pal, Tyra Brown, Jinping Yang, Suimin Qiu, Susan McCammon, Gracie Vargas
The University of Texas Medical Branch at Galveston, Galveston, TX

Background: Detection of oral and oropharyngeal cancer (>600,000 new cases/year worldwide) relies on visual inspection and palpation for biopsy guidance, an approach that despite increased prevention/screening efforts results in a low unchanging 5-year survival rate. A method providing indicators of neoplasia parallel to histology could improve biopsy guidance. Label-free multiphoton autofluorescence microscopy (MPAM) has been shown to indicate cellular, extracellular, and architectural atypia in animal models of neoplasia. MPAM was evaluated for the ability to identify atypia in human neoplasia.
Study Design/Materials and Method: Clinical normal and neoplastic samples were obtained from sites of the oral and oropharyngeal mucosa of subjects undergoing therapeutic resections for lesions or tumours. Samples were imaged by MPAM with parameters for the redox fluorophores at sites indicated to be clinically normal, tumour, and transition sites. Imaged sites were processed for H&E and immunohistochemistry for metabolic markers (glut-1). Age, smoking status, EGFR, HPV status, and gender were obtained. Categorical analysis was performed of cellular and morphometric variables with pathological grading as the standard.
Results: Sites obtained included floor of the mouth, oropharynx, tongue, and lip, with the first two comprising the majority of sites > 17 subjects. MPAM allowed visual identification of cellular and extracellular atypia in neoplasia and redox potential measures were possible. Quantitative measures showed statistical significance between normal and neoplasia ($p < 0.01$) in all sites with collagen atypia most evident in larynx.
Conclusion: MPAM applied in human neoplasia showed benefits similar to those of previous animal model assessments, identifying cellular and extracellular atypia associated with neoplasia. Additional correlates with metabolic and biological variables are ongoing and will also be discussed.

NOVEL DEVICE FOR *IN VIVO* HNSCC MARGIN DETECTION

Peter A. Pellionisz, Karam W. Badran, Zachary E. Taylor, Harrison Cheng, Warren S. Grundfest, Maie St. John
David Geffen School of Medicine at UCLA, Los Angeles, CA; Henry Samueli School of Engineering and Applied Sciences, UCLA, Los Angeles, CA

Background: The major causes of oral, head and neck cancer-related deaths are persistent or recurrent disease. The primary management of advanced-stage oral, head and neck cancer relies on the complete surgical resection of the tumour. However, the establishment of a histologically sound, margin-free resection is often difficult given the devastating side effects of aggressive surgery and the anatomic proximity to structures

such as the carotid artery and the spinal cord. Severe consequences may include loss of voice (laryngeal cancers), swallowing and speech problems (tongue cancers), and anatomic deformities (orbit, palate etc.). Unfortunately, positive margin status, commonly defined as any viable tumour at the edge of the resected tumour, is associated with significantly decreased survival due to local or regionally persistent or recurrent disease. Despite focused research in conventional therapies, the five-year survival rate for patients with advanced oral cancer remains approximately 15–20 percent. Therefore, early detection or prevention of this disease is likely to be most effective. The absence of definite early warning signs, or validated biomarkers for oral cancers suggests that a handheld optical tool would be critical for screening high risk patients.
Study Design/Materials and Method: Oral squamous cell carcinoma specimens and surrounding tissues from the surgical bed were collected; fluorescence decay images were acquired using a wide field DOCI system. Samples (55 patients) were subsequently processed for standard histological assessment by head and neck pathologists. Mean relative fluorescence decay signatures were calculated for tumour, fat, muscle and collagen tissues. Statistical analyses were performed using the Wilcoxon signed rank test.
Results: DOCI produces pixel values that are proportional to the aggregate fluorophore of the probed tissue without the requirement of fitting acquired data to complex mathematical models. Qualitative analysis of DOCI images revealed microscopic characterization sufficient for tissue type identification comparable to histology. Quantitative analysis revealed a statistically significant difference ($p < 0.05$) between tumour and collagen among ten of ten wavelength bands analyzed, between tumour and muscle in ten bands, and between fat and tumour in two bands.
Conclusion: This study demonstrates a novel imaging modality capable of rapidly and significantly distinguishing OSCC from surrounding normal tissue. Such an intraoperative tool would be transformative: allowing for an intraoperative capacity to delineate tumour tissue from non-tumour tissue, thus maximizing the efficacy of tumour resection and minimizing damage to adjacent structures, thus improving patient outcomes.

OPTICAL COHERENCE TOMOGRAPHY AND LASER SPECKLE IMAGING OF THE BRAIN THROUGH A TRANSPARENT CRANIAL IMPLANT IN A CHRONIC MOUSE MODEL

David L. Halaney, Carrie Jonak, Nami Davoodzadeh, Junze Liu, Jasmine Shah, Pasha Ehtiyatkar, Hyle Park, Devin Binder, Guillermo Aguilar
University of California Riverside, Riverside, CA

Background: Optical diagnostic and therapeutic neuro-procedures are hindered by the highly scattering cranial bone, necessitating craniotomy (i.e. removal of a section of skull) to provide optical access each time a procedure is performed. Repeated cranial surgeries are expensive and increase risk to the patient. We have proposed a solution to this issue previously by introducing a novel transparent cranial implant made of nanocrystalline yttria-stabilized zirconia (nc-YSZ), which we call the Window to the Brain (WttB) implant.
Study Design/Materials and Method: In our previous work, we demonstrated that imaging depth and contrast of optical coherence tomography (OCT) imaging is improved through the WttB implant compared to the native skull in an acute murine model. In this present work, we extend this acute study with OCT performed through the WttB implant immediately following cranioplasty, and at multiple subsequent time points over 30 days in a chronic murine implant model. Additionally, we present complementary laser speckle imaging blood flow mapping across the WttB implant over corresponding time points.
Results: Factors influencing the stability of this model, such as cranial bone regeneration and changes to implant transparency due to fibrotic deposition and/or tissue adhesion will be discussed.
Conclusion: This work represents the next step towards a viable transparent implant for chronic use, and demonstrates the potential value of this murine implant model as a tool to evaluate the neurological effects of new drugs, procedures, or to study neurological physiology and disease longitudinally over time in a single animal.

RAPID EMPIRICAL CHARACTERIZATION OF SUB-DIFFUSE REFLECTANCE IMAGING FROM MOHS SURGERY SKIN SAMPLES

Will Goth, Xu Feng, Katherine Sebastian, Jennifer Vickers, Matthew Fox, Jason Reichenberg, James W. Tunnell
The University of Texas at Austin, Austin, TX; Seton Healthcare Family, Austin, TX

Background: Spatial Frequency Domain Imaging (SFDI) is a label free imaging technique which is sensitive to tissue microstructure. Recent studies have used SFDI in the sub-diffuse regime (sd-SFDI), where the influence of absorption is minimized and the signal is predominantly sensitive to differences in cellular and nuclear structure relevant to tumour pathology. As abnormal microstructure is the primary diagnostic indicator in Mohs micrographic surgery (MMS), sd-SFDI provides the opportunity for tumour identification without sectioning and staining the sample, a significant bottleneck in MMS workflows. However, the processing time for existing non-linear sd-SFDI models needs further improvements to realize near real-time image rendering. Here, we present a linearized empirical model of sub-diffusive scattering and apply it to sf-SFDI data from optical phantoms and human MMS skin samples.
Study Design/Materials and Method: Optical phantoms of polystyrene microbeads [$d \approx 0.1$–$1.0\,\mu m$] at varying reduced scattering coefficients [$\mu s' \approx 1$–3 mm-1] were imaged at high spatial frequencies [$f > 0.5$mm-1], corresponding to the tissue sub-diffuse scattering regime. Excised human MMS skin samples were imaged and compared to accompanying H&E stained histology, which were examined by expert histopathologists. An existing non-linear model and our new linearized empirical model were fit to collected sd-SFDI data.
Results: Our empirical model coefficients showed high sensitivity to changes in scattering particle concentration and size in the optical phantoms. The model parameters highlighted regions of basal cell carcinoma tumour, epidermis, dermis, fat, and other features on the human skin cancer samples. These parameters were additionally used to segment the MMS samples, and corresponded well with the regions demarcated by histology. Our linearized model required less than 1 second to process 1 million pixels, whereas the non-linear model required over 5 hours.
Conclusion: The proposed empirical model is a rapid and powerful tool to quantify sd-SFDI data without the need for extensive modeling and fitting, reducing the processing time of existing non-linear models by several orders of magnitude. The high sensitivity of our model to different microstructural constituents in experimental results provides a potential path to a streamlined MMS workflow.

VISUALIZATION OF LASER-ASSISTED DRUG PERMEATION IN NAIL TISSUE

Vinzent K. Ortner, Peter A. Philipsen, Merete Haedersdal, Jonathan R. Brewer
Bispebjerg Hospital, Copenhagen, Denmark; DaMBIC, University of Southern Denmark, Odense, Funen, Denmark

Background: This study is aimed at exploring the potential of enhanced drug delivery in the nail using laser ablation and advanced optical microscopy to observe potentially altered drug behavior. Compared to skin, nail plates show distinctly different tissue reaction in response to ablative laser treatments. Consequently, all aspects of existing knowledge on cutaneous laser-assisted drug delivery are not directly transferable and need to be reevaluated in a nail-focused context to further evolve this technique. Coherent anti-stoke Raman spectroscopy (CARS), confocal fluorescence microscopy (CFM), and multiphoton microscopy (MPM) are valuable tools to visualize fluorescent drugs and structures of the nail.

Study Design/Materials and Method: Healthy nail clippings were collected and had a single fractional CO_2-device laser treatment with 5% density at 40 mJ fluence. The fluorescent drug analog ATTO-647N was applied to the top surface of both the laser-porated and untreated control samples. Cryotome-sectioning and multimodal imaging of the samples were performed at hourly time points after labeling.

Results: Untreated samples showed high signal intensity, from the drug analog, along the top layer of the nail surface and nearly no penetration into the nail. The laser-porated nails demonstrated penetration of the drug analog into the nail. A clear buildup of the drug analog at the bottom layer of the nail, with approximately 80% of the maximum top layer signal intensity, was seen, resulting in a double peak signal intensity distribution.

Conclusion: This study demonstrated the value of incorporating optical imaging to explore laser-assisted drug delivery to the nail. Multimodal microscopy visualized the drug's enhanced diffusion as well as its locally selective deposition in laser-porated nails. By gaining deeper insight into differences between cutaneous and ungual drug delivery, laser-assisted drug delivery could be expanded to become a topical treatment option for various nail diseases.

BASIC SCIENCE AND TRANSLATIONAL RESEARCH – *IMAGING I: IMAGE BASED MONITORING*

DOPPLER OPTICAL COHERENCE TOMOGRAPHY WITH A VORTEX BEAM

Jason B. King, Eun Kim, Daniel M. Santos, Nitesh Katta, Thomas E. Milner
University of Texas at Austin, Austin, TX

Background: Doppler optical coherence tomography (DOTC) has been used to measure velocity of moving particles – such as red blood cells in vasculature. The Doppler shift is due to particle movement along the direction of beam propagation causing a time-varying phase shift in the recorded signal. We examine use of DOTC using laterally phase structured light – a vortex beam – with an azimuthal phase variation. We simulate and apply DOCT with a vortex beam to measure angular velocity.

Study Design/Materials and Method: A computer simulation including a vortex beam interacting with an arbitrary number of rotating scattering structures was completed. The simplest case of one rotating scatterer was considered first. We created models with multiple scatterers and different angles between the beam propagation direction and axis of rotation. For each case, we unwrapped OCT spectral phase corresponding to different vortex beam modes and angular velocities. We have also tested this approach experimentally using a spatial light modular to generate a vortex beam in an OCT system. Simulated and experimental results were generated and compared.

Results: For the model with one scatterer, the unwrapped spectral phase over time was directly proportional to the angular velocity and vortex beam mode number, and did not depend on location of the scatterer. With multiple scattering centers, this was true only when the angular velocity and beam propagations vectors were co-aligned. When angular velocity and beam propagation vectors are misaligned, unwrapped phase also depended on the location of the scatterers and matches to what we have observed experimentally.

Conclusion: The models show that a vortex beam may be used to measure angular velocity by analyzing the unwrapped spectral phase of the OCT signal, although the analysis becomes more challenging with multiple scatters with both angular and longitudinal movements.

INTRAOPERATIVE TISSUE TEMPERATURE MONITORING BASED ON SPECKLE OPTICAL COHERENCE TOMOGRAPHY DURING CUTANEOUS LASER THERAPY

Mary Sheu, Yong Huang, Sewon Kang, Jin U. Kang
Johns Hopkins School of Medicine, Baltimore, MD

Background: Cutaneous laser surgery involves selective tissue heating and resulting thermal effects on specific targets with minimal collateral damage to surrounding tissues. The extent and specificity of tissue damage is dependent on peak tissue temperature, the laser exposure time, and the target tissue characteristics. Current laser treatments rely largely on clinical endpoints to determine proper laser settings. Clinical endpoints however may be difficult to gauge, are subjective and depend on the monitoring skill of the operator. Therefore, the ability to monitor the tissue temperature in real-time during the laser therapy would allow for an objective and accurate assessment of the tissue region under treatment.

Study Design/Materials and Method: Optical coherence tomography (OCT) is a fast, non-invasive imaging modality that can provide 3D information of biological tissues. OCT images contain fully developed speckles that carry important information on microscopic scatter motion within the tissue, which can be correlated to temperature variation. A 1310 nm swept source based OCT system with 50,000 A-scans/s imaging speed was used on both phantom (floppy disk film) and *ex vivo* biological tissue to evaluate the temperature sensing capability.

Results: A phantom radiation of laser-pulse train from 9 μJ to 200 μJ showed that Speckle OCT can track different temperature rising amplitude with millisecond resolution. In addition, inter-frame speckle decorrelation analysis of OCT imaging of resected from human skin showed gradual temperature rise during laser treatments.

Conclusion: Speckle information from real-time fast OCT imaging, provided effective monitoring of tissue temperature during laser surgery with high spatial and temporal resolution. The ability to accurately monitor skin temperature in real time would result in safer, more consistent laser delivery, which may lead to better clinical outcome.

MONITORING NANOSECOND PULSED LASER THERMAL DYNAMICS IN TISSUE USING OPTICAL COHERENCE TOMOGRAPHY

Nitesh Katta, Austin McElroy, Thomas E. Milner
University of Texas at Austin, Austin, TX

Background: Thermography for pulsed laser surgery is sometimes essential to limit non-specific thermal damage along with monitoring the tissue real time. Conventional techniques including thermocouples and IR imaging are limited by either their point sampling methodologies or surface images often with no depth resolved feedback. Imaging techniques with depth information used to monitor these processes often lack the spatiotemporal resolutions to obtain useful feedback. Swept source based optical coherence tomography (OCT) measurements has the potential to provide a real-time depth-resolved image with relevant spatiotemporal sampling characteristics to guide laser photothermal surgeries.
Study Design/Materials and Method: We present an optical coherence tomography (OCT) based relative thermography methodology for studying the dynamics of surgical laser treatment. For this, a 15W nanosecond pulsed thulium (Tm) 1940 nm fiber laser was first integrated with a swept source laser (1310+/− 70 nm). The measurements were carried out to monitor the responses of the pulsed Tm irradiation. OCT based relative thermography (OCRT) was used to investigate this response through enhanced decorrelation techniques. The technique's feasibility was initially tested in Polydimethylsiloxane (PDMS) phantoms. The methodology was applied *in vivo* murine brains to monitor the coagulation process of blood vessels, and generate relative thermographic contrasts.
Results: OCT thermography was able to detect thermally active regions, and successfully predict the thermal relaxation time in a depth resolved setting in PDMS phantoms. The method was then used to monitor the thermal relaxation process in *ex vivo* tissue sections of pig skin. The methodology was subsequently applied to monitor the coagulation process *in vivo* of blood vessels in the murine brain caused by irradiation of the pulsed Tm 1940 nm fiber laser onto the tissue.
Conclusion: The methodology successfully predicted thermal relaxation times in PDMS phantoms in response to pulsed thulium laser irradiation. Given the small footprint of OCT devices, usage of this technique to monitor and guide pulsed laser surgeries appears feasible for *in vivo* applications.

MULTIPHOTON MICROSCOPY OF COLLAGEN STRUCTURE IN *EX VIVO* HUMAN SKIN FOLLOWING ELECTROCHEMICAL THERAPY

Allison C. Hu, Ellen Hong, Wesley Moy, Tatiana Krasieva
Beckman Laser Institute and Medical Clinic, University of California, Irvine, CA; University of California, Irvine, Orange, CA

Background: Injury to the dermis and dermoepidermal junction initiates a robust healing process consisting of collagen deposition and scar formation. Management of scars and other skin injuries relies on local soft tissue transfer, resurfacing and/or graft placement, and are invasive, exacerbate integumentary injury, or expensive (lasers). In this study, we examine the creation of *in situ* redox generated pH changes in fresh human skin. We believe this process, "electrochemical therapy" (ECT), leads to changes in collagen matrix structure. Our objective is to map local tissue pH landscapes and image changes in collagen structure using ECT.
Study Design/Materials and Method: Remnant fresh *ex vivo* human facial skin from facelift operations was enveloped in saline soaked gauze (two hours) prior to ECT and imaging. ECT was performed by inserting platinum-plated needle electrodes connected to a DC power supply. Voltage (4, 5, or 6) and time (3, 4, or 5 min.) were varied systematically. High frequency ultrasound (25 MHz) was performed immediately after ECT on each sample. Second harmonic generation (SHG) microscopy visualized collagen fiber. pH landscapes were mapped using indicator dyes in bisected specimens. The SHG images were compared with histopathologic findings.
Results: Above 4 V and 3 minutes, a profound reduction in dermal collagen SHG signal was observed at the anode. Less SHG signal loss was observed at the cathode, and a decrease at the dermoepidermal junction was observed instead. pH maps suggest ECT spatial selectivity and a direct relationship between voltage and application time. Ultrasound demonstrated a direct relationship between dosimetry and structural changes.
Conclusion: ECT alters tissue pH leading to dermal collagen structural change. Findings here suggest ECT may be a simple and low cost approach to locally remodel the soft-tissue matrix. Future directions aim to expand into a skin injury model to determine if similar collagen effects are observed *in vivo*. ECT is low cost (~$5) and may be a means to treat soft tissue injuries using simple needle based devices and DC battery power supplies.

OPTICAL POLARIZATION IMAGING FOR GUIDING SKIN CANCER SURGERY – A PILOT TRIAL

Peter R. Jermain, Xin Feng, Victor Neel, Anna N. Yaroslavsky
University of Massachusetts Lowell, Lowell, MA; Massachusetts General Hospital, Boston, MA

Background: The aim of this study was to evaluate wide-field optical polarization imaging (OPI) for preoperative delineation of nonmelanoma skin cancers. Currently, surgeons visually assess the extent of tumour spread in the skin. Visual assessment is prone to errors involving insufficient or excessive removal of tissue. By mapping disruptions in dermal collagen network in real time, OPI has the potential to provide an accurate tool for identifying the lateral extent of cancer prior to surgery.
Study Design/Materials and Method: This pilot clinical study was performed at the Massachusetts General Hospital under an IRB-approved protocol. In total, we have imaged forty-eight subjects with fifty-three biopsy confirmed nonmelanoma skin cancer (NMSC), scheduled for Mohs surgery. Prior to excising the cancer, the study surgeon outlined the lesion with blue marker, which indicated the borders of the initial excision. Cross-polarized reflectance images were acquired at 440 nm and 640 nm. 440 nm visualized collagen network, whereas 640 nm visualized surgeon's blue marker. Following image acquisition, routine Mohs surgery was performed. The optical images were evaluated against the gold-standard of histopathology, routinely processed during Mohs surgery.
Results: Out of fifty-three lesions imaged, forty-three were basal cell carcinomas (BCC) and ten were squamous cell carcinomas (SCC). All 10 SCCs were negative for cancer after the first stage. Out of 43 BCCs imaged, 13 had positive margins after the first excision. All 13 were positive in the lateral

dimension. In all thirteen cases, cancer margin assessment by the OPI correlated well with histopathology. Out of 30 cases negative after the first stage, OPI correlated with histopathology in each case.
Conclusion: These results indicate that OPI shows promise for pre-surgical evaluation of tumour margins, and holds the potential to help reduce the number of surgical stages needed to completely remove the tumour and spare normal tissue.

SPATIAL FREQUENCY DOMAIN IMAGING FOR BURN WOUND ASSESSMENT: A CASE SERIES

Anna-Marie Hosking, Gordon Kennedy, Rebecca Rowland, Melissa Baldado, Ata Sharif, Amaan Mazhar, Adrien Ponticorvo, Nicole Rouse, Nicole Bernal, Anthony J. Durkin
Beckman Laser Institute and Medical Clinic, University of California, Irvine, CA; Modulated Imaging Inc., Beckman Laser Institute Photonic Incubator, Irvine, CA; University of California – Irvine, Medical Center, Orange, CA
Background: Timely and accurate determination of burn wound depth is imperative as it dictates the treatment regimen and thus, clinical outcome. The most widely employed method for determining burn depth and severity is bedside clinical evaluation, which is a highly subjective science. Even among experts in the field, diagnosis has been reported to be accurate only two-thirds of the time. Early excision and grafting of deep-partial and full thickness burns has been shown to improve functional and aesthetic outcomes, while decreasing complications such as infection, contractures, and hypertrophic scarring. As such, accurate and timely burn wound assessment and intervention is of significant clinical importance. Spatial frequency domain imaging (SFDI) is a unique, wide-field imaging modality based on diffuse optical spectroscopic principles and is well-suited for quantitative imaging of tissue. SFDI is able to quantitatively capture information related to collagen denaturation (via scattering changes), hemodynamics, and vascular damage, each of which provides clinicians with objective means to assess burn wound severity.
Study Design/Materials and Method: After obtaining informed consent, we prospectively evaluated 3 burn patients from a regional burn center with SFDI in addition to standard care and evaluation by a burn surgeon.
Results: Our preliminary results show that SFDI is capable of measuring *in vivo* parameters such as blood oxygen saturation and characterizing scattering changes to differentiate superficial, superficial-partial, deep-partial, and full thickness burns as early as day one after injury. Additional cases will be accrued and analyzed in the final results.
Conclusion: This is the first study to describe the use of SFDI in the *in vivo* evaluation of burn patients, providing clinicians with objective means to assess burn wound severity.

THE USE OF OPTICAL COHERENCE TOMOGRAPHY IN THE ASSESSMENT OF ACNE-LIKE INFLAMMATORY LESIONS IN RESPONSE TO A TOPICAL MINOCYCLINE GEL TREATMENT ON A MOUSE MODEL

Maiko Hermsmeier, Tanvee Sawant, Khadiza Chowdhury, Jun Hyung Sin, Xin Chen, Usha Nagavarapu, Kin F. Chan
BioPharmX, Menlo Park, CA
Background: Current successful outcomes of clinical studies for acne vulgaris depend greatly on achieving primary endpoints with statistically significant reduction in acne lesion count and improvement in Investigator's Global Assessment (IGA) score of the drug product against its vehicle control. As there is no validated preclinical model for this indication, an acne-like inflammatory lesion mouse model was developed to provide guidance and confidence for drug screening in our pharmaceutical development programs. In this study, the model was used to assess the efficacy of a topical minocycline gel, in conjunction with optical coherence tomography (OCT) as an assessment tool to monitor the response of acne-like lesions to topical treatment.
Study Design/Materials and Method: Live P. acnes were injected intradermally resulting in acne-like inflamed lesions similar to those observed in the clinic. Topical minocycline gel was applied to these lesions once daily for two weeks along with vehicle and untreated control groups, during which the lesion progression was monitored and measured with both microcaliper and OCT. While microcaliper is a common tool used in preclinical studies, it is labor intensive and can be prone to subjectiveness. In this study, we concurrently acquired OCT images across the *apex* of these cylindrically symmetrical lesions, generating more accurate width (B-scan) and height (A-scan) measurements, which were used to calculate lesion volume. Volume measurements from the two methods were statistically analyzed.
Results: A general decrease in lesion appearance was observed qualitatively through OCT cross-section images, and a general decrease in lesion volume was observed quantitatively in lesions of the topical minocycline treatment group compared to those in both the untreated and vehicle control groups.
Conclusion: Our findings support the use of OCT in evaluating lesions in preclinical studies, which could potentially lead to less subjective examination in the clinic, yielding more accurate and less time-consuming results.

BASIC SCIENCE AND TRANSLATIONAL RESEARCH: *LIGHT-BIOFILM INTERACTIONS*

ANTIMICROBIAL BLUE LIGHT INACTIVATION OF MICROBIAL CLINICAL ISOLATES IN BIOFILMS

Raquel Ferrer-Espada, Yanyan Fang, Ying Wang, Tianhong Dai
Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA
Background: Biofilms cause more than 80% of infections in humans and are extremely resistant to antimicrobials and the immune system. The situation is exacerbated by the fast spreading of antimicrobial resistance, which has become one of the biggest threats to current public health. There is consequently a critical need for the development of alternative therapeutics. Antimicrobial blue light (aBL), is a light-based approach that, opposed to antimicrobial photodynamic therapy (aPDT), exhibits intrinsic antimicrobial effect without the involvement of exogenous photosensitizers. In this study, we investigated the effectiveness of this non-antibiotic approach against biofilms formed by clinical isolates of multidrug-resistant bacteria and Candida albicans.
Study Design/Materials and Method: Clinical isolates of multidrug-resistant Acinetobacter baumannii, Escherichia coli,

Neisseria gonorrhoeae, Pseudomonas aeruginosa, Proteus mirabilis, and C. albicans were studied. Biofilms were grown in microtiter plates or in the CDC biofilm reactor for 24 or 48 hours and exposed to aBL at 405 nm. The anti-biofilm activity of aBL was measured by viable counts.

Results: Our results indicated that while the biofilms of all species were susceptible to aBL inactivation to some extent, N. gonorrhoeae and P. aeruginosa showed the most susceptibility with 4 and 6 log reductions respectively after 108 J/cm^2 (60 mW/cm^2, 30 min) and 216 J/cm^2 (60 mW/cm^2, 60 min) aBL were delivered in the microplates. On the other hand, the thickness of the biofilm of C. albicans made it the least susceptible microorganism to aBL inactivation.

Conclusion: aBL exhibits great potential against pathogenic microorganisms and could help with the significant need of new antimicrobials in the clinical practice to manage multidrug resistant infections.

ANTIMICROBIAL BLUE LIGHT INACTIVATION OF NEISSERIA GONORRHOEAE: IMPLICATIONS FOR THE TREATMENT OF MULTIDRUG-RESISTANT GONOCOCCAL INFECTIONS

Ying Wang, Raquel Ferrer Espada, Leon G. Leanse, Tianhong Dai, Ying Gu

Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA; Chinese PLA General Hospital, Beijing, China

Background: Gonorrhea is the second most prevalent sexually transmitted infection globally. Its etiological bacterium, Neisseria gonorrhoeae, has developed resistance to nearly every antibiotic ever used for treating it. CDC considers multidrug-resistant N. gonorrhoeae as an urgent threat. It's critical to devise alternative strategies. This study aimed to evaluate the efficacy of an innovative non-antibiotic approach, antimicrobial blue light (aBL), for inactivating N. gonorrhoeae.

Study Design/Materials and Method: The anti-N. gonorrhoeae efficacy of aBL (405 nm) was evaluated using five multidrug-resistant N. gonorrhoeae strains (ATCC700825 and four clinical isolates). Mechanism of aBL was analyzed by identifying the endogenous photosensitizers with LC-MS, verifying the involvement of singlet oxygen by singlet oxygen specific quencher (NaN3), and observing the ultrastructure changes induced by aBL using TEM.

Results: All the five strains were highly susceptible to aBL. To induce a 3-log10 CFU reduction in planktonic suspensions, 36 J/cm^2 aBL exposure was required for ATCC700825 and 36–72 J/cm^2 for the four clinical strains. To achieve a complete eradication of the N. gonorrhoeae CFU (>8-log10 CFU reduction), 54 J/cm^2 aBL was needed for ATCC700825 and 72–108 J/cm^2 for the four clinical strains. In the biofilms formed by the clinical isolate #179, a 4-log10 CFU reduction was achieved when 108 J/cm^2 aBL was delivered. HPLC revealed the presence of endogenous porphyrins and flavins in N. gonorrhoeae. Singlet oxygen played a vital role in the anti-N. gonorrhoeae effect of aBL. TEM images showed that aBL increased the electronic density of both cytoplasm and nucleoid DNA.

Conclusion: Present results indicated that aBL is a potential strategy to combat multidrug-resistant gonococcal infections. Further studies are warranted in treating genital tract gonococcal infections in mice and exploring potential clinical applications.

ESTABLISHING A SAFETY WINDOW FOR LASER GENERATED SHOCKWAVE TREATMENT FOR BACTERIAL BIOFILMS

William Yao, Young Hun Chung, Edward C. Kuan, Maie St. John, Warren S. Grundfest, Zachary D. Taylor

University of California: Los Angeles, Los Angeles, CA; Ronald Reagan UCLA Medical Center, Los Angeles, CA

Background: Bacterial biofilms are frequently found in chronically infected wounds. Biofilms prevent effective penetration of antibiotics, and can often only be treated with surgical debridement. Laser generated shockwave (LGS) therapy is a novel tissue-sparing treatment for biofilm disruption. Previous studies have shown the effectiveness of LGS in disrupting biofilms *in vitro*. In this study, we aim to determine the safety threshold of the LGS technology in an *in vivo* rodent model.

Study Design/Materials and Method: The dorsal skin of Sprague-Dawley rats were shaved, waxed, and a treatment area covering 4×2 cm^2 was treated with LGS at three different peak pressures: 118, 296, and 227 MPa. Settings for LGS were based on a 1064 nm Nd:YAG laser (pulse duration 9 ns) with laser fluence of 777.9 mJ. Peak pressures were controlled using laser spot size of 2.2, 3.0, and 4.2 mm diameters, respectively. Following treatment, skin samples were excised and assess for tissue injury or inflammation under histology. Each treatment group consisted of 9 rats ($n = 3$/each for 1-hour, 24-hour, 72-hour post-treatment). An additional 4 control (untreated) rats were included in the analysis, for a total of 31 animals.

Results: Gross injuries occurred in 21 (77%) rats and consisted of minor erythema, with prevalence positively correlated with peak pressure ($p < 0.05$). All injuries under gross observation resolved within 24 hours. Under histological analysis, all injuries and inflammation were found to be localized to the epidermis and superficial dermis.

Conclusion: LGS appears to be well tolerated by cutaneous tissue for the laser energy settings shown to be effective against bacterial biofilm *in vitro*. All injuries incurred, at even the highest peak pressures, were clinically mild and resolved within 1 day. This lends further support to the overall safety of LGS and serves to translate LGS towards *in vivo* efficacy studies.

LASER GENERATED SHOCKWAVE TREATMENT INCREASES BACTERIAL CELL MEMBRANE PERMEABILITY *IN VITRO*

Young Hun Chung, William Yao, Edward C. Kuan, Maie St. John, Warren S. Grundfest, Zachary D. Taylor

University of California of Los Angeles, Los Angeles, CA

Background: Chronically infected wounds cause pain, loss of function and mobility, and increased healthcare burdens. Biofilms protect the underlying bacteria in these wounds and current methods of debridement can be damaging to underlying tissue. Laser generated shockwave (LGS) therapy is a novel debridement approach that can increase permeabilization of bacterial biofilm for better antibiotic therapy. In this study, we investigate, through flow cytometry, if LGS can permeabilize bacterial cell membranes.

Study Design/Materials and Method: The study utilized propidium iodide (PI) for the flow cytometer dyes, as PI permeates damaged cell membranes. Five solutions containing planktonic S. epidermidis underwent LGS treatment with a 1064 nm Nd:YAG laser (laser fluence of 110.14 mJ/mm^2, pulse duration 9 ns, spot size 3 mm) with dye input times ranging

from 0 to 20 minutes at 5 minute intervals. One solution each of all live (positive) or all dead (negative) non-treated controls were included for comparison. The solutions were treated once in microcentrifuge tubes with shockwaves generated from laser ablation of titanium on polyimide substrates. Filters at 561 nm and 488 nm were used to analyze the cells under a SORP BD LSRII Analytic Flow Cytometer.
Results: The solution with non-treated live cells showed little absorption of PI, but all treated solutions showed stronger absorbance levels. 20 minutes after LGS treatment, the cells still demonstrated persistent uptake of PI. The dead cells showed the highest level of PI absorbance.
Conclusion: The results suggest that LGS may have a direct permeabilization effect on the bacterial cells. The low PI values for the non-treated live cells and higher PI values for the treated cells support this. Future studies need to assess the impact of multiple treatments to the bacteria. This data confirms that LGS can be a potentially useful adjunct to the treatment of bacterial biofilms in chronic wound and soft tissue infections.

BASIC SCIENCE AND TRANSLATIONAL RESEARCH: *PHOTOBIOMODULATION*

EFFECT OF PHOTOBIOMODULATION ON AN EXPERIMENTAL MODEL OF CHRONIC ASTHMA: PARTICIPATION OF LEUKOTRIENES

Auriléia A. Brito, Karina Brajato, Veridiana Delle, Clariana Rodrigues Soares, Stephanie S. Soares, Ana Paula L. Oliveira
Universidade Nove de Julho, São Paulo, Brazil

Background: Studies leukotriene receptor antagonists, such as montelukast (MK) has contributed at different levels for the treatment of asthma. Our studies indicate that LLL therapy is effective in reducing allergic inflammation and pulmonary remodeling in an experimental model of lung diseases. Thus, the present study aimed to evaluate the effects of LLL therapy on an experimental model of ovalbumin-induced chronic pulmonary allergic inflammation (OVA).
Study Design/Materials and Method: BALB/C mice were divided into four groups: Basal, LLL, OVA, OVA + LLL. Chronic lung allergic inflammation was induced by ovalbumin sc (OVA) immunization, mixed with alum (days 0 and 14) and oral tracheal challenge with OVA (three days/week for five weeks). The OVA + LLL group was irradiated (diode laser, 660 nm, 30 mW, and 3J). Twenty four hours after the last treatment, the animals were anesthetized, tracheotomized, cannulated and the bronchoalveolar lavage was collected and analyzed (total and differential cell counts as well as cytokine levels in the bronchoalveolar lavage through the elisa technique).
Results: Both treatments reduced the total number of cells and eosinophils in bronchoalveolar lavage (BAL) ($p < 0.001$). However, in the simultaneous treatments the reduction was more significant ($p < 0.01$). There was a significant reduction ($p < 0.05$). In IL-5 levels of all treated groups, IL-13 levels in the treated groups were significantly reduced ($p < 0.001$). Regarding leukotrienes, we noticed a significant decrease ($p < 0.05$) in LTB4 levels in the OVA + LLL group in relation to the OVA group. There was a reduction in the deposition of collagen and mucus fibers in the airways in the treated group ($p < 0.001$), in the evaluation of pulmonary mechanics, a significant decrease ($p < 0.001$) was observed in the treated group, in all situations evaluated.
Conclusion: Thus, these results indicate that is effective in reducing allergic inflammation, remodeling and pulmonary elastance. These effects appear to be mediated by modulation of the LLL in the secretion of the leukotrienes.

EFFICACY OF PHOTOBIOMODULATION THERAPY IN MITIGATING SKIN RADIATION DAMAGE

Rodrigo Crespo Mosca, Sofia Nascimento Santos, Gesse Eduardo Calvo Nogueira, Daisa Lima Pereira, Francielli Campos Costa, Praveen Arany, Carlos Alberto Zeituni
Nuclear and Energy Research Institute, Radiation Technology Center, São Paulo, Brazil; Oral Biology & Biomedical Engineering School of Dental Medicine, Engineering & Applied Sciences, University at Buffalo, Buffalo, NY

Background: The use of sophisticated radiation dose delivery and fractionation has significantly improved cancer care. One of these involves localized, sustained ionizing dose delivery termed brachytherapy. Despite it therapeutic efficacy, specific side effects of brachytherapy include localized skin damage and breakdown for which only palliative treatments are currently available. The use of low dose biophotonics treatments to promote tissue healing is termed photobiomodulation (PBM) therapy. The aim of this study was to evaluate efficacy and molecular pathways of PBM therapy using two common wavelengths, red and near-infrared (NIR) to treat radiation wounds in athymic mice subjected to brachytherapy (sustained ionizing radiation from 125I seeds).
Study Design/Materials and Method: A pilot study was performed with thirty-six athymic mice were accomplished for 60 days and divided into six groups: Surgical Control Group (No radiation and no PBM treatments); Radiation Control Group (125I seed 0.4252 mCi, no PBM); NIR-PBM Control Group (NIR PBM alone, LED at $\lambda = 880$ nm); Red-PBM Control Group (Red PBM alone LED at $\lambda = 660$ nm); Radiation- NIR PBM Group; Radiation-Red PBM Group. Following 21 days, radiation-induced wounds are evident. PBM treatments (both wavelengths with output power 40 mW for 20 s, fluence 20 J/cm^2 on top of implantation site) were performed every week up to 60 days. Wounds were evaluated every 7 days digital imaging, Laser Doppler Flowmetry (LDF) and tissue temperature with a thermographic camera. We also performed μPET-CT imaging using radioactive fluorodeoxyglucose (18F-FDG) at 51 and 81 days post-implantation. Animals were sacrifices progressively at each time point to correlate clinical observations with imaging and molecular tissue analyses. Tissues were collected to analyze molecular pathways correlating with inflammation, immune response, wound healing and angiogenesis using mRNA (qRT-PCR) and protein expression (immunostaining).
Results: Both PBM treated groups demonstrated significant ($p < 0.05$) improvements in skin radiation wound healing as compared to radiation group. Distinct improvements in clinical wound size and closure, improved tissue perfusion and reduced inflammation as evidenced by decreased wound thermal images. These wounds were also noted to have significant differences in the cytokine profiles (TGF-β, VEGF and PDGF) correlating with better healing responses. Radiation damage reduces brown fat composition that can potentially contribute to additional radiation-associated morbidities. The μPET-CT imaging noted significant preservation of brown fat composition in PBM-treated

radiation alone groups. Further validation of these pathways is ongoing.
Conclusion: Within the parameters of this study, PBM treatments demonstrated improved healing in radiation wounds due to ionizing radiation from 125I seeds. Ongoing work is examining the precise molecular pathways contributing to these therapeutic benefits. It is hoped this study will enable further development of this innovative therapy for managing side-effects from radiation treatments.

ELECTROMYOGRAPHIC EVALUATION OF MOTOR RESPONSE TO PHOTOBIOMODULATION FOR THE TREATMENT IN PATIENTS WITH SPINAL CORD INJURY

Andréa O. Gomes
Universidade Nove de Julho, São Paulo, Brazil

Background: Traumatic spinal cord injury can range from mild medullary concussion to transient dormancy and permanent quadriplegia. The most common sites of this lesion are at the level of the cervical vertebrae, C5, C6 and C7 and at the level of the thoracic and lumbar vertebrae, T12 and L13. The literature is unclear as to an effective therapy to improve the quality of life of patients with spinal cord injury. Photobiomodulation is a promising resource in musculoskeletal injuries, so the role of this study is to assess the potential of phototherapy in patients with sensorimotor deficit.
Study Design/Materials and Method: This study involves 25 patients with a diagnosis of spinal cord injury recruited at the Physiotherapy Clinic of the University of Nove de Julho (UNINOVE, Brazil), who underwent 12 sessions of phototherapy with electromyographic evaluation at the beginning and at the end of the sessions. The irradiation was administered to the site of injury transcutaneously using a *Quantum* diode laser (Ecco Fibers and Devices, Brazil) with wavelength of 808 nm, aperture diameter 0.18 cm, Irradiance at aperture 4.72 W/cm, Number of 5 points irradiated, area irradiated of the 0.0254 cm and frequency of treatment sessions of the twelve sessions were performed on three per week for four weeks. The EMG signals were captured using a four-channel acquisition system (EMG 432C, EMG System do Brazil Ltda.) consisting of a signal conditioning module, bipolar active electrodes, analog band pass filter from 20 to 500 Hz and common mode rejection ratio of 120 dB. The sampling frequency was 2 kHz, scanned using analog/digital (A/D) conversion board with 16 bits of resolution.
Results: In the phototherapy group, median frequency values of the brachial biceps and femoral quadriceps muscles were higher at rest and during isotonic contraction 30 days after photobiomodulation ($p = 0.0258$). No significant results were found regarding to the rest and isotonic conditions in the pre-photobiomodulation period ($p = 0.950$) or immediately following photobiomodulation ($p = 0.262$).
Conclusion: The data provide evidence that phototherapy improves motor response in individuals with spinal cord injury through the difference before and after treatment with phototherapy.

INCREASED MITOCHONDRIAL METABOLISM BY TWO DIFFERENT WAVELENGTHS DID NOT RESULT IN ALTERED FIBROBLASTIC PROLIFERATION *IN VITRO*

Juanita J. Anders, Xingjia Wu, Ilko K. Ilev
Uniformed Services University of the Health Sciences, Bethesda, MD; Food and Drug Administration, Silver Spring, MD

Background: Previous photobiomodulation (PBM) studies on *in vitro* fibroblasts reported increased cellular proliferation. Typically these culture models had decreased serum levels in the media so that growth conditions were not optimal. The purpose of this study was to determine if combinations of irradiance (mW/cm^2) and time resulting in different fluences (J/cm^2), for different wavelengths, that were effective in altering mitochondrial metabolism as measured by the MTS assay also resulted in cellular proliferation using adult human dermal fibroblasts (HDF) grown in high glucose medium.
Study Design/Materials and Method: The laser wavelengths used were: 810 nm and 980 nm. A precision light delivery device which included an electronic shutter was used. Adult HDF were cultured in chamber slides (10,000 cells/well) with defined medium (control) or the control medium with high glucose (180 mM). Cells in high glucose medium were treated with the following laser parameters which we previously identified as significantly altering mitochondrial metabolism: 50, 100, 200, and 300 mW/cm^2 and 0.05, 0.2, 1, 5, 20 J/cm^2. All settings were replicated four times. Twenty-four hours after laser treatment, cells were washed and frozen at −80 °C overnight. The CyQuant assay was applied to measure the DNA-based proliferation. Statistical analysis was done using one way ANOVA with Tukey multiple comparison test.
Results: Although all the laser parameters used had significantly increased mitochondrial metabolism in the HDF, this effect did not result in increased cellular proliferation in this high glucose *in vitro* model.
Conclusion: It is commonly stated in the literature that PBM results in improved cell survival, increased proliferation and migration, and new protein synthesis implying that all these results occur after PBM therapy. This data demonstrates that the secondary effects that occur after light absorption and increased mitochondrial activity depends on the state of the cell and are dictated by the physiological needs of the cell.

LOW LEVEL LASER THERAPY INHIBITS NEUROPATHIC PAIN AND RESTORES THE MORPHOLOGICAL PATTERN OF THE SCIATIC NERVE OF DIABETIC MICE

Victória da Silva Oliveira
University of São Paulo, São Paulo, Brazil

Background: Diabetic peripheral neuropathy (DPN) is one of the most common complications caused by diabetes mellitus and the development of chronic pain is the most prevalent symptom. Conventional treatments for DPN are still unsatisfactory, leading to the search for new therapies. Low level laser therapy (LLLt) arises as a new alternative target, through its analgesic, anti-inflammatory and biomodulators effects inducing a significant improvement of disabilities observed on DPN. Herein we evaluated the therapeutic potential of LLLt in a model of diabetic neuropathy induced by streptozotocin (STZ) in mice, as well as possible mechanisms involved in its effect.
Study Design/Materials and Method: Male, C57BL/6 mice (20–26 g; 8 weeks old − CEUA-ICB 22/2014) were used thought this study. Animals received a single injection of STZ (225 mg/kg i.p.) and, after 14 days, mechanical hypersensitivity was confirmed by von Frey filaments. LLL was applied to the left hind paw (1.6 J/cm^2; 30 mW; 15 secs, spot size 0.28 cm^2) for 21 consecutive days. Behavioral testing was repeated after 7, 14 or 21 LLL-sessions. Sciatic nerves were removed at the 21st day for nerve growth factor (NGF) evaluation by ELISA and for morphology evaluation by transmission electronic microscopy. Behavioral tests were analyzed by two-way ANOVA followed by Bonferroni's post-test; NGF quantification was analyzed by nonparametric t test. The significance level considered was $p < 0.05$.

Results: LLLt induced antinociception in neuropathic pain mice (n = 12) when compared to the control group (n = 10). An increase on NGF levels in the sciatic nerves of LLL-mice (n = 5) was also observed. Morphometric analysis demonstrated a decrease on axoplasma-myelin separation (n = 6; 42.75%) of LLL-treated mice as well as a substantial improvement in myelin and an increase in the number of cytoplasmic mitochondria.
Conclusion: LLLt reverses neuropathic pain of diabetic mice by restoring NGF levels and inducing regeneration of sciatic nerve.

TGF-β AND TNF-α CROSSTALK IN MEDIATING MACROPHANGES RESPONSES TO PHOTOBIOMODULATION THERAPY IN BURN WOUND HEALING

Saeed Ur Rahman, Praveen Arany
School of Dental Medicine, University at Buffalo, Buffalo, NY

Background: Successful tissue repair needs efficient functional activities of immune cells such as neutrophils and monocyte-macrophages. Macrophages have a prominent role in the wound healing processes. They disinfect wounds by eliminating microbes, engulfing and digesting cellular debris and other foreign substances. TGF-β has been shown to be an essential wound cytokine with its several effects on most cell populations including increasing migration of Monocyte-Macrophages, wounded keratinocytes, endothelial cells. Previously, we had noted the ability of PBM treatments to activate latent TGF-β1. Photobiomodulation (PBM) therapy is the use of low dose biophotonics devices to reduce pain and inflammation and improve wound healing. However, the exact mechanism of photobiomodulation on macrophage biology is yet to be fully explored. The aim of this study was to investigate the anti-inflammatory effects of PBM therapy on the proliferation, migration, and phagocytic activity of macrophage *in vitro*.
Study Design/Materials and Method: A murine macrophage cell line, Raw 264.7 cells were treated with a bacterial cell wall component, lipopolysaccharides (LPS) to simulate infection. We used two PBM clinical treatment wavelengths namely, blue (450 nm) and red (650 nm) LED and examined their effects on proliferation, migration, and phagocytic activity of macrophages. LED treatments were carried out for 100 seconds and varying irradiances (10–150 mW/cm^2) to deliver effective dose (irradiances from 1–10 J/cm^2). A fluorescence assay, Alamar blue, was employed to determine cell proliferation at 24 hours post-treatment. Cell migration was examined by scratching (wounding) a confluent cell layer and assessing their ability to move towards each other over 24 hours. Finally, macrophages function assessed by phagocytosis of latex beads following PBM therapy was investigated using fluorescence microscopy and quantitative image analysis.
Results: Our results noted that PBM therapy significantly increased proliferation of macrophages with both blue and red right at specific doses. As treating these cells with recombinant TGF-β also increased proliferation, pre-incubation with a small molecule inhibitor against TGF-β (SB431542) mitigated the PBM proliferative effects. We then examined the effects of PBM therapy in the presence of LPS that reduced macrophage proliferation. PBM therapy with both wavelengths demonstrated increased survival fraction in LPS treated macrophages. Cell motility in macrophages are a key aspect of their function in wounds. PBM treatments appeared to promote macrophage migration in the *in vitro* wound assay with blue LED treatments appearing to be more effective. Interestingly, TGF-β1 and its inhibitor (SB431542) had antagonistic effects with inhibition and stimulation of motility respectively. PBM treatments in the presence of the TGFβ inhibitor stimulated the macrophage motility further. Moreover, treatments with LPS increased macrophage motility that was further stimulated by PBM treatments. Finally, we noted the ability of PBM treatments to promote phagocytic activity of macrophages that was stimulated by TGF-β inhibitor. This was even more evident in cells treated LPS treatments that could be stimulated further with blue compared to red PBM treatments. Based on the contradictory effects of PBM activated TGF-β in these assays, we investigated the crosstalk of other inflammatory signaling pathways in these responses. Given the central role of the TNF-α signaling pathway in mediating several of these macrophage responses, we examined the effects of PBM treatments on TNF-α expression using an ELISA and promoter assays. We observed that PBM treatments reduces TNF-α levels in macrophages. Ongoing work is assessing the role of TGF-β and TNF-α signaling crosstalk in mediating the macrophage responses.
Conclusion: Overall, these results demonstrate that PBM therapy is able to promote a more efficient inflammatory response by evoking a robust macrophage response that results in better tissue healing. These observations are particularly relevant in enabling optimal clinical translation of PBM therapy in compromised wounds such as burn, infected or diabetic wounds. In conclusion, PBM therapy offers a novel clinical tool to promote wound healing by therapeutically modulating the inflammatory response.

BASIC SCIENCE AND TRANSLATIONAL RESEARCH: *PRECLINICAL THERAPEUTICS*

EVALUATION OF CELL DEATH MECHANISMS CAUSED BY CONDUCTIVE POLYMER NANOPARTICLE-MEDIATED PHOTOTHERMAL THERAPY

Madeline E. Huff, James W. Tunnell, Jennifer Irvin, Tania Betancourt
Texas State University, San Marcos, TX; University of Texas at Austin, Austin, TX

Background: Nanoparticle-mediated photothermal therapy (PTT) has recently been a highly explored approach in the field of nanomedicine for the localized treatment of cancer through the conversion of near infrared radiation into thermal energy. While extensive PTT research has been performed using gold nanoshells and gold nanorods, conductive polymer nanoparticles (CPNPs) have only recently been explored as alternative agents for laser-induced ablation of the target tumour tissue. The synthesis, characterization, and *in vitro* evaluation of cell death mechanisms using CPNPs for PTT is described.
Study Design/Materials and Method: CPNPs were prepared from the monomer 3,4-ethylenedioxythiophene (EDOT) using a two-surfactant oxidative-emulsion polymerization process, yielding poly (3,4-ethylenedioxythiophene) (PEDOT) NPs. PEDOT NP characterization was carried out by UV/VIS/NIR absorption spectroscopy, dynamic light scattering, zeta potential analysis, transmission electron microscopy and scanning electron microscopy. Temperature change as a function of nanoparticle concentration and laser irradiation time was verified using an 808 nm laser at 2.17 W/cm^2 and a thermocouple. Calcein Blue AM, Annexin V AlexaFluor 488, and Propidium Iodide were utilized as biochemical indicators on cells after exposure to PEDOT NPs and irradiation with the

NIR laser in order to determine the mechanisms of cell death by fluorescence microscopy.
Results: CPNPs have a strong absorbance in the NIR region, with a peak absorbance at 790 nm, an average diameter of 70 nm, and a zeta potential of roughly −30 mV. The 100 and 500 μg/ml NP concentrations displayed prominent cell death by the apoptosis Annexin V marker after 1.5 hours of NP exposure. After 24 hours of NP exposure, cells displayed an increase in propidium iodide fluorescence, which is a marker of necrosis.
Conclusion: The results of this work demonstrates that poly (3,4-ethylenedioxythiophene) NPs are effective as photothermal agents for the ablation of cancer. Low NP-cell exposure times expressed more apoptotic cell death, while higher NP-cell exposure times expressed an increase in necrotic cell death.

EVALUATION OF SCARRING AND HEALING IN NO-ADHESIVE NIR LASER TISSUE BONDING

Vidyasagar Sriramoju, Stewart Russell, Robert Alfano
The City College of New York, New York, NY

Background: Laser tissue bonding (LTB) in the near infrared (NIR) without the use of exogenous adhesive can produce scar-free wound closure without complications related to failure or biological incompatibility of the adhesive. Comparison of different methods of LTB against sutured closure to evaluate postoperative scarring is required to advance this technology to the clinic.
Study Design/Materials and Method: We compare continuous wave (CW) and femtosecond pulsed (fs) no-adhesive laser tissue bonding to sutured wound closure, by evaluation of gross histology and microscopic evaluation of H&E and Massons trichrome stained histological sections. *In vivo* experiments on guinea pig were conducted with CW (lambda = 1455 nm, power = 200 mW) and fs (lambda = 1560 nm, power − 95 mW, pulse width = 150 fs) lasers against sutured controls, and evaluated postoperatively.
Results: Gross histology revealed that, at 42 days post operation, the fs welded tissue exhibited almost normal epidermis with no hyperkeratosis, minimal collagen deposition, a normal surface contour, and minimal loss of dermal appendages. The CW laser bonded tissues exhibited near normal epidermis with minimal hyperkeratosis, minimal collagen deposition, near normal surface contour, and minimal loss of dermal appendages.
Conclusion: Both CW and fs produce less scar formation and improved healing over sutures in both gross and microscopic histological examination. In addition, fs laser showed improved healing and scarring response relative to the CW laser.

FEASIBILITY OF A NOVEL POLYCHROMATIC NEAR-INFRARED LASERS DIODE − AN *IN VIVO* SAFETY STUDY

Orit Topaz, Yaron Assaf, Joseph Lepselter
Alma Lasers Ltd., Caesarea, Israel; Asymmetric Medical Ltd, Hertzeliya, Israel

Background: Near-infrared lasers (755, 810, 1064 nm) are widely used for decades in procedural dermatology, each has its unique skin optical signature, typically with high safety and efficacy profile. However, the laser-tissue interaction of a new lasers diode emitting simultaneously 3 near-infrared wavelength (755*810*1064 nm) is unknown. We used a porcine model to *in vivo* assess the safety of a novel polychromatic near-infrared lasers diode and compared it to monochromatic near-infrared wavelength lasers diode.
Study Design/Materials and Method: Domestic female crossbred pigs were exposed to 4 different lasers diode wavelength: 755, 810, 1064 nm and 755*810*1064 nm. Laser energy levels were 20J (low), 60J (medium) and 200J (super high). Skin biopsies (H&E) were taken 30-min after radiation at day 0, 3 and 10. Similarly, spleen and muscle were radiated and biopsied in order to assess for coagulation.
Results: Transient erythema was evident in all technologies at super high levels though its severity varied. 1064 nm diode that triggered the highest rise in temperature (41 °C), caused the most severe erythema whilst 810 nm diode caused the mildest one (34 °C). The 755*810*1064 nm HP caused lower damage compared to 1064 nm diode and similar to 755 nm diode HP. At day 0, no changes were detected in the epidermis and stratum corneum in all 4 lasers diode across energy levels. The only histologic changes detected were blood congestion at high power levels and a focal streaming of nuclei in the epidermis. Moreover, 755*810*1064 nm coagulated the spleen and muscle in similar to the other laser diodes. At 3 and 10-day skin samples were compared to the controls. Epidermis and dermis showed no abnormal structural changes. There were no histological differences between the control and the treatment samples.
Conclusion: The 755*810*1064 nm lasers diode presents similar safety profile as monochromatic near-infrared lasers diode technology. Its clinical merit in procedural dermatology is yet to be determined.

FLUORESCENCE IMAGING AND PHOTOTHERAPY OF BREAST CANCER USING ERYTHROCYTE-DERIVED NEAR INFRARED NANOPARTICLES

Joshua Burns, Raviraj Vankayala, Jenny Mac, Bahman Anvari
University of California − Riverside, Riverside, CA

Background: We have engineered nano-sized vesicles derived from erythrocytes loaded with indocyanine green (ICG) as a potential light-activated theranostic agent. We refer to these constructs as near infrared (NIR) erythrocyte-derived transducers (NETs) since once photo-excited by NIR light they can transduce the energy to emit fluorescence, generate heat, or produce reactive oxygen species (ROS). The objective of this study was to demonstrate the utility of NETs in mediating photo-destruction of breast cancer (SKBR3) cells *in vitro* and in a mouse tumour model, and investigate the mechanism of photo-destruction.
Study Design/Materials and Method: We used fluorescence imaging and flow cytometry to examine NETs uptake by cells, ROS production and cellular death in response to 808 nm irradiation. We explored the theranostic capabilities of NETs in an *in vivo* mouse model consisting of SKBR3 implanted tumours. We used whole body fluorescence imaging following intravenous injection of NETs and irradiated tumours at 808 nm for 10 minutes at power densities of 340 or 680 mW/cm^2. To investigate the photothermal and/or photochemical mechanism of tumour destruction, we made subcutaneous temperature measurements using a thermocouple placed ≈ 2 mm away from laser irradiated tumour, and used caspase 3 immunostaining to assays the extent of apoptosis.
Results: NETs exhibited high intracellular uptake and produced ROS leading to cellular death. Our *in vivo* experiments demonstrated visualization of tumours through fluorescence imaging. Sub-cutaneous temperature rises on the order of ≈ 11°C were measured in response to irradiation at

680 mW/cm^2. Apoptosis was induced in response to both applied power densities.
Conclusion: Our results indicate that NETs provide a capability for NIR fluorescence imaging of cancer cells and implanted tumours in mice. Photothermal and photochemical mechanisms as medicated by NETs can induce destruction of cancer cells and tumours.

FRACTIONAL LASER-ASSISTED DELIVERY OF TOPICAL BLEOMYCIN – QUANTIFICATION AND IMAGING WITH MASS SPECTROMETRY

Kristoffer K. Hendel, Charlotte Bagger, Uffe Høgh Olesen, Christian Janfelt, Steen Honoré Hansen, Merete Haedersdal, Catharina M. Lerche
Bispebjerg Hospital, Copenhagen, Denmark

Background: Bleomycin exhibits antiproliferative effects desirable for use in dermato-oncology, but its molar mass of 1415 Da prevents topical application. Ablative fractional laser (AFXL)-assisted drug delivery has been shown to enhance drug uptake in skin. The objective is to quantify delivery of topical bleomycin after AFXL-exposure and to visualize biodistribution in skin.
Study Design/Materials and Method: In an *in vitro* Franz diffusion cell study, pig skin samples (n = 66) were laser-exposed with 0, 5, 20 or 80 mJ/microbeam at 5% density prior to application of a bleomycin (11,250 IE = 5295 μg) or saline solution. The samples were exposed to bleomycin or saline for 0.5, 4 or 24 hour(s). Bleomycin was quantified from biopsy cryosections at depths of 100, 500, and 1500 μm using high-performance liquid chromatography-mass spectrometry (HPLC-MS), and its biodistribution visualized using matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI).
Results: AFXL-exposure enhanced bleomycin delivery to superficial skin after 0.5 hours of drug exposure and to all skin depths after 4 hours with the 20 and 80 mJ/mb settings ($p < 0.05$). Controls receiving no laser exposure were mainly below level of quantification. Drug uptake depended on laser channel depth. Thus, after 24 hours, the 5 mJ/mb setting delivered 1533.2 IE/cm^3 (722 μg/cm^3) bleomycin into the superficial dermis (100 μm) and 1083 IE/cm^3 (510 μg/cm^3) into the deep dermis (1500 μm). In comparison, use of the 80 mJ/mb setting enhanced delivery to 3522.3 IE/cm^3 (1658.7 μg/cm^3) and 2336 IE/cm^3 (1100 μg/cm^3) at 100 and 1500 μm, respectively ($p < 0.05$). MALDI-MSI qualitatively visualized biodistribution and revealed high uptake in the laser-channel coagulation zones with notable delivery in the surrounding skin tissue.
Conclusion: AFXL-assistance greatly enhances topical bleomycin uptake with high deposition in the coagulation zones and surrounding skin tissue. Using higher AFXL-pulse energy levels enhances bleomycin uptake and penetration depth.

LASER-ASSISTED DELIVERY OF A SYNERGISTIC COMBINATION CHEMOTHERAPY IN *IN VIVO* SKIN

Emily Wenande, Joshua Tam, Brijesh Bhayana, William A. Farinelli, Steven Kyle Schlosser, Emily Ishak, Agata Chlopik, Mai P. Hoang, Omar R. Pinkhasov, Peter Caravan, R. Rox Anderson, Merete Haedersdal
Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA; Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark

Background: The chemotherapeutics cisplatin and 5-fluorouracil (5-FU), display synergistic activity against epithelial tumours. For non-melanoma skin cancer however, topical efficacy is marred by insufficient deep skin penetration. This study investigates ablative fractional laser (AFL)-assisted cisplatin and 5-FU delivery, examining impact on pharmacokinetics, biodistribution and toxicity in *in vivo* skin.
Study Design/Materials and Method: Up to 60 min topical cisplatin and 5-day 5-FU exposure were assessed alone or in combination in intact and CO_2 fractional laser-exposed skin of two swine. From 1–120 hours, cisplatin and 5-FU were detected at 500-1500μm skin depths using mass-spectrometry. Drug quantification in plasma at 0–120 hours assessed systemic exposure, while evaluation of local skin toxicity comprised clinical scores and transepidermal water loss (TEWL) measurement. Quantitative evaluation of apoptosis (TUNEL) and epidermal proliferation (ki67) and qualitative analysis of histologic skin sections assessed histological effects.
Results: AFL altered pharmacokinetics of cisplatin and 5-FU, leading to rapid uptake in deep skin layers. Two hours after application, cisplatin was enhanced up to 5-fold versus 5-hour control skin, with peak concentrations increasing from 5.09 μg/cm^3 to 29.3 μg/cm^3 at 1500 μm depth ($p = 0.002$). Correspondingly after 1hr, 5-FU deposition at 500 μm exceeded levels requiring 5-day exposure in intact skin. In the deep compartment, no 5-FU was detected before 120 hours in non-laser-exposed samples (1500 μm, 17.67 μg/cm^3), contrasting to AFL's 18-fold enhancement by 1 h (318.74 μg/cm^3, $p = 0.001$). Neither drug was detected in plasma from 0–120 hours. Clinical scores and TEWL in sites exposed to combination delivery were consistently among the most elevated ($p = 0.002$). Congruently, combined delivery more than doubled apoptosis versus AFL alone, greater than separate drug delivery at 48 hours ($p < 0.014$). AFL + cisplatin + 5-FU further caused significant reduction in epidermal proliferation versus AFL + cisplatin ($p < 0.001$), with an earlier nadir than AFL + 5-FU. H&E revealed neutrophilic infiltrate and confluent epidermal necrosis in AFL + 5-FU and AFL+ cisplatin + 5-FU samples after 120 hours, most pronounced with combination delivery.
Conclusion: AFL provides enhanced and deeper early cisplatin and 5-FU uptake, with intensified clinical and histological effects after combination drug delivery.

MOUSE MODEL OF COLD-INDUCED LOCALIZED FAT LOSS (SELECTIVE CRYOLIPOLYSIS)

Nunciada Salma, Michael Evers, Malte J. Casper, Christine Droigk, Timo Kepp, Heinz Handels, Dieter Manstein
Massachusetts General Hospital, Charlestown, MA; University of Lübeck, Lübeck, Schleswig-Holstein, Germany; Institute for Medical Informatics at the University of Luebeck, Schlewig-Holstein, Germany

Background: Localized, non-invasive cold exposure (selective cryolipolysis) is able to induce a localized loss of fatty tissue and is already used clinically. Selective Cryolipolysis is a method based on the observation that fat cells are more susceptible to cold-induced injury as compared to other tissues.
Study Design/Materials and Method: We used C57BL/6J mice as a model for localized cold exposure. Mice were anesthetized, shaved and tattooed in the inguinal area. One side of the inguinal area was exposed to cold using a

thermal-control-cooling probe for 10 minutes at −10°C while the other side was not exposed to cold (control area). Subcutaneous fat thickness was determined by optical coherence tomography (OCT) at day 7 and day 30 after treatment and was quantified using a custom-made-machine-learning algorithm. Histological evaluation of the skin and inguinal fat pads was also performed.
Results: We observed a reduction of ~20–30% of subcutaneous fat thickness after a month compared to the unexposed control area. Histologically cold induced panniculitis in mice and localized fat loss was induced and quantified.
Conclusion: This mice model of cold-induced localized fat loss can help to further investigate the mechanism of selective cryolipolysis and to further improve clinical performance by investigating the role of medication and adjuvants taken with the procedure.

TEMPERATURE DEPTH PROFILES INDUCED IN HUMAN SKIN *IN VIVO* USING PULSED 532, 975, AND 1064 nm IRRADIATION

Matija Milanic, Adam Cenian, Nina Verdel, Witold Cenian, Jost Stergar, Boris Majaron
University of Ljubljana, Ljubljana, Slovenia; Polish Academy of Sciences, Szewalski Institute of Fluid-flow Machinery, Gdansk, Poland; Jozef Stefan Institute, Ljubljana, Slovenia

Background: The aim of this study was to determine the temperature depth profiles induced in human skin *in vivo* by a pulsed 975 nm diode laser (5 ms pulse length) and compare them to those induced by more common KTP (532 nm) and Nd:YAG (1064 nm) lasers.
Study Design/Materials and Method: Measurements were performed on volar sides of the forearms in eight volunteers with healthy skin (type I–II). At irradiation spot diameters of 4–5 mm, the radiant exposures were approximately 0.36, 0.30, and 5.4 J/cm^2 for the KTP, diode, and Nd:YAG lasers, respectively. Temperature depth profiles induced in human skin by all three lasers were determined using pulsed photothermal radiometry (PPTR). This technique involves time-resolved measurements of mid-infrared emission from the irradiated test spot and reconstruction of the laser-induced temperature profiles using an earlier developed optimization algorithm.
Results: After normalization of the reconstructed temperature profiles to a radiant exposure level of 1 J/cm^2, the maximal temperature rise in the epidermis averaged at 10.8 K, 0.8 K, and 0.4 K for the KTP, diode, and Nd:YAG lasers, respectively. Similarly, average maximal dermal temperature rises induced by the same lasers were 4.0 K, 0.6 K, and 0.2 K. Meanwhile, the depths up to which 50% of the absorbed laser energy was deposited were 0.33 mm, 0.47 mm, and 0.57 mm (for the KTP, diode, and Nd:YAG lasers). The assessed relations and temperature depth distributions were successfully reproduced in a numerical simulation of light transport in human skin, based on the multiple-layer Monte Carlo technique and known spectral behavior of the main skin absorbers (i.e., melanin, blood, lipids, and water) and scattering coefficients.
Conclusion: Energy deposition characteristics of the millisecond 975 nm diode laser are very suitable for controlled heating of the upper dermis, as required for non-ablative skin rejuvenation. The risks of overheating the epidermis or subcutis are significantly reduced in comparison with 532 nm or 1064 nm irradiation.

MECHANICAL ASSESSMENT OF A DEVICE DEDICATED TO INTRAOPERATIVE PHOTODYNAMIC THERAPY FOR GLIOBLASTOMA TREATMENT

Clément Dupont, Nicolas Reyns, Pascal Deleporte, Serge R. Mordon, Maximilien Vermandel
Univ. Lille, Inserm, CHU Lille, U1189 – ONCO-THAI – Image Assisted Laser Therapy for Oncology, F-59000 Lille, France

Background: Glioblastoma Multiforme (GBM) is a malignant brain tumour with an incidence of at 3–5 for 100,000 persons. GBM prognosis remains poor (median survival below 15 months) even with a complete standard of care including surgery, radiation oncology and chemotherapy. Relapse occurs in 85% of cases in the 5 mm margin of the resection cavity. In this context, photodynamic therapy (PDT) delivered intraoperatively may be a relevant treatment option to improve local control.
Study Design/Materials and Method: A lighting device was designed to fit to the resection cavity and illuminate the surrounding brain tissues. The behavior of the lighting device within the surgical cavity (pressure against the healthy parenchyma, conformity to the cavity) was studied by mean of a specific phantom with similar brain mechanical properties. A gel has been poured inside a 3D printed mold, enabling to obtain the same shape and mechanical properties of a patient's brain with a surgery cavity, previously segmented on an intraoperative MRI sequence. Once the device placed inside the cavity, CT-scan examinations were achieved.
Results: By delineating each slice of the CT volume, a conformation index was computed from the ratio of the area where the balloon touches the brain's phantom to the area of the cavity. This coefficient was approximately equal to 32%. Despite of this value appears to be weak, but the dosimetric model associated to the device remains relevant.
Conclusion: In this study, we present a mechanical assessment of a lighting device used in intraoperative PDT for GBM treatment and currently evaluated in the phase one INDYGO clinical trial. Although the conformation of the device remains modest, the dosimetric model is still acceptable. Nevertheless, further investigations should be achieved to quantify this impact using several Monte-Carlo modeling of cavities imaged with intraoperative MRI.

CLINICAL APPLICATIONS – CUTANEOUS: *ACNE*

A RANDOMIZED SPLIT-FACE CLINICAL TRIAL COMPARING 1550 nm ERBIUM-DOPED FRACTIONAL LASER AND 755 nm ALEXANDRITE PICOSECOND PULSE DURATION LASER WITH DISTRACTIVE LENS ARRAY IN THE TREATMENT OF ATROPHIC ACNE SCARS

Mahsa Karavan, Erika M. Summers, Payam Tristani, Mark B. Taylor, David Smart
University of Utah, Salt Lake City, UT; Revere Health Dermatology, Provo, UT; Gateway Aesthetic Institute & Laser Center, Salt Lake City, UT

Background: Acne scarring has detrimental impact on a person's mental and social well-being, thus more effective therapies with fewer side effects are needed. More recently 1550 nm erbium-doped fractional photothermolysis system (FPS) has shown great efficacy and safety in the treatment

of acne scarring. Following the advent of 755 nm Alexandrite Picosecond laser (APL), few studies have shown significant improvement in acne scarring with minimal side effects using a fractionated handpiece. To date there has been no study comparing these lasers. Our aim is to compare the efficacy and safety of 1550 nm fractional erbium-doped laser and 755 nm APL with diffractive lens array.

Study Design/Materials and Method: We conducted a randomized double blind split-face, prospective study of 22 Fitzpatrick skin type I–V patients with moderate to severe atrophic acne scarring. Participants were randomized to receive 3 one-month interval treatments of FSP on one side of the face and APL on the other side, with a 3 month follow up. Primary outcome was patient reported satisfaction and side effect profile. Secondary outcome was improvement in scarring as assessed by 3 blinded physician evaluators. A tertiary objective outcome of scar improvement is reported using VISIA complexion analysis system.

Results: Overall there were no statistically significant differences in the patient-reported and blinded-reviewer improvement of acne scarring comparing the 1550 nm fractionated and 755 nm alexandrite picosecond lasers. However subjects reported statistically significant fewer side effects and lower pain on the side treated with APL (81% vs. 18%, $p = 0.005$). Either laser demonstrated no lasting complications among all Fitzpatrick skin types.

Conclusion: In conclusion we found no clinical difference in scar improvement between the two lasers and overall less patient-reported side effects with APL. Therefore, alexandrite picosecond laser could be used as an equally efficacious treatment of atrophic acne scars with a superior pain and side effect profile as compared to FPS.

CLINICAL EVALUATION OF A MICRONEEDLING DEVICE WITH RADIOFREQUENCY FOR THE TREATMENT OF ACNE SCARS IN ETHNIC SKIN

Omar A. Ibrahimi

Connecticut Skin Institute, Stamford, CT

Background: The treatment of acne scars in skin of colour remains a challenge. Here, we evaluate the safety and efficacy of a microneedling device with radiofrequency for the treatment of acne scars.

Study Design/Materials and Method: A retrospective, single center study evaluating the safety and efficacy of a microneedling device with radiofrequency was performed. All subjects with Fitzpatrick Skin Types IV–VI that received one or more treatments over two consecutive years were analyzed. Clinical efficacy was quantified using a blinded physician evaluator using a commonly accepted quartile grading system for laser tattoo removal. The occurrence of adverse events were also recorded.

Results: 13 subjects met the study criteria, including subjects of Asian, South Asian, Hispanic and Black ethnic descent. Treatment efficacy data is awaiting completion of data analysis but demonstrates the utility of microneedling with radiofrequency in treating acne scars in skin of colour. No adverse events were noted to persist beyond one month.

Conclusion: Microneedling with radiofrequency is safe and effective for treating acne scars in skin of colour. This technology extends the therapeutic options for the treatment of acne scars and the low incidence of adverse events in ethnic skin is unique.

LACK OF LONG-TERM IMPROVEMENT IN ACNE SCARRING AFTER MICRONEEDLING

Georgina M. Ferzli, Daniel A. Belkin, Hana Jeon, Roy G. Geronemus

Laser and Skin Surgery Center of New York, New York, NY

Background: Microneedling has gained popularity as a treatment modality for acne scarring. Though several studies have shown promise in the efficacy of microneedling for acne scars, few studies have evaluated long-term outcomes of this treatment. In this study, we examine the results of microneedling alone for acne scarring at both 6 weeks and 18 weeks post-final treatment.

Study Design/Materials and Method: Eighteen patients with acne scarring were treated with microneedling to the bilateral cheeks, using a 1.5 mm dermaroller to achieve pinpoint bleeding. Patients received three treatment sessions at 3-week intervals. Patients returned for follow-up visit at 6 weeks and 18 weeks post-final treatment. Photographs were taken at screening and both follow-up visits. Three blinded, board-certified dermatologists were asked to evaluate side-by-side images and identify the improved image, if change was noted. Results comparing screening versus final follow-up, screening versus 6-week follow-up, and 6-week follow up versus 18-week follow-up were tabulated and analyzed.

Results: When analyzing side-by-side images of screening visit versus 18-week follow-up visit, blinded evaluators chose the follow-up image as the improved image only 34% of the time. When comparing the 6-week follow-up visit to the screening visit and to the 18-week final follow-up, evaluators chose the 6-week follow-up image as the improved image 59% and 52% of the time, respectively. When evaluating the images of the 6-week follow-up visit versus final follow-up visit, the evaluators identified the long-term follow-up image as improved only 16% of the time.

Conclusion: This evaluator-blinded study demonstrates that the effects of microneedling on acne scarring are transient. Evaluators were unable to identify the 18-week follow-up images as improved when compared to screening images and short term follow-up images in the majority of patients. Nevertheless, evaluators did recognize improvement in scarring at 6-week follow-up when compared to both baseline and final follow-up images, suggesting a temporary and transient improvement in the appearance of acne scars after microneedling treatment.

LASER PRE-CONDITIONING AS NEW METHOD FOR 1726 nm SELECTIVE PHOTOTHERMOLYSIS OF SEBACEOUS GLANDS

Fernanda H. Sakamoto, William A. Farinelli, R. Rox Anderson

Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA

Background: 1726 nm is an absorption band of sebum lipids with about 30% higher absorption than water. This minimal contrast poses a challenge to achieve selective photothermolysis of sebaceous glands without injury to the overlying epidermis.

Study Design/Materials and Method: Human and porcine skin samples *ex vivo* were exposed to a 1726 nm fiber laser using 2.5 mm spot, 100–200 ms, at up to 40W laser power. Skin samples were placed on a warm plate at 37 °C, with a cold sapphire window at − 5 °C in contact with the skin

surface. A thermal camera placed orthogonally was used to image temperature distribution within the skin before and during laser exposures. With a single laser pulse, there was nearly equal heating at the skin surface, and the mid-dermis where sebaceous glands exist. A strategy of preconditioning was devised to provide bulk heating of the mid-dermis prior to delivery of the selective photothermolysis laser pulse. A series of lower power laser pulses were delivered for 1–20 seconds prior to the treatment laser pulse. Nitroblue tetrazolium (NBTC) staining was used to assess cell viability, and histology to assess skin injury.

Results: Laser preconditioning of the skin for 20 seconds at 0.5 W, followed by a single treatment pulse of 30–35 W produced consistent thermal damage a depth of 0.5–1 mm, while consistently sparing the epidermis. NBTC showed epidermal viability. Histology showed selective thermal damage to sebaceous glands.

Conclusion: The strategy of laser pre-conditioning is a new way to enhance selective photothermolysis for dermal targets. When there is minimal absorption contrast, such as for sebaceous glands at 1726 nm, pre-conditioning is necessary. The strategy may be applicable for other dermal targets of selective photothermolysis.

TREATMENT OF ACNE SCARRING WITH A NOVEL DUAL-WAVELENGTH LASER

Michael H. Gold, Serge R. Mordon

Tennessee Clinical Research Center, Nashville, TN; INSERM U1189 & Lille University, Lille, France

Background: Facial acne scarring is a prevalent disease with both physical and psychosocial sequelae. An innovative solid state dual wavelength 1319 nm and 589 nm laser, which does not require consumable dye, was investigated for the treatment of acne scars.

Study Design/Materials and Method: A total of 12 patients (11 female, 1 male – Fitzpatrick skin phototypes II & III) with acne scar for more than one year, were treated with 1319 nm and subsequently by 589 nm, all having three-sessions, one every other week. A full face was covered in approximately 30 minutes. Acne scars were scored by one physician or sub-investigator using the ECCA grading scale before and 2 weeks after treatment. Safety was measured by recording subject discomfort scores and adverse effects.

Results: Fluence used was $28\,J/cm^2 \pm 2.4\,J/cm^2$ at 1,319 nm and $16 \pm 2.9\,J/cm^2$ at 589 nm. At baseline, mean ECCA score was 99 ± 21. This score was reduced after each session to 85 ± 27 ($p < 0.05$), to 67 ± 18 ($p < 0.01$) after 2 sessions, to reach 58 ± 15 ($p < 0.005$) after the 3rd session. This observation corresponds respectively to 16%, 34% and 42% reduction of the ECCA score. Only one patient (ECCA score: 120) did not improve after 3 sessions. Slight to moderate erythema was sometimes observed without dryness or bruising. No or minimal burning or stinging was reported. No crust was observed. 6 month follow-up is scheduled.

Conclusion: Improvement in scarring was noted in almost all patients with minimal discomfort and minimal downtime. Combining both minimal side effects with effective acne scar reduction, this laser appears to be highly effective. Long-term evaluation remains necessary to confirm the efficacy of this new laser.

TREATMENT OF FACIAL ACNE SCARS WITH MICRONEEDLING IN COMBINATION WITH POLYMETHYLMETHACRYLATE (PMMA)

Brian S. Biesman, Joel L. Cohen, Barry E. DiBernardo, Jason J. Emer, Roy G. Geronemus, Michael H. Gold, Todd E. Schlesinger, Craig F. Teller

Brian Biesman, MD, Nashville, TN; Joel Cohen MD, Greenwood Village, CO; Barry DiBernardo MD, Montclair, NJ; The Roxbury Institute, Beverly Hills, CA; Laser and Skin Surgery of New York, New York, NY; Tennessee Clinical Research Center, Nashville, TN; Todd E. Schlesinger, MD, Charleston, SC; Bellaire Dermatology Associates, Bellaire, TX

Background: Microneedling and PMMA injections in combination may provide even greater efficacy than either modality alone for the treatment of facial acne scars. A randomized, multicenter trial assessed the efficacy and safety of microneedling alone versus microneedling followed by PMMA (Suneva Medical, San Diego, CA) for correction of atrophic facial acne scars.

Study Design/Materials and Method: Subjects ($n = 45$) with acne scars of all severities were treated with microneedling alone and followed for 12 weeks (Period 1). At week 12 subjects were randomized to treatment with PMMA or no treatment and followed for 12 weeks (Period 2). Results were evaluated at the end of Period 1 and Period 2 using the 5-point Acne Scar Assessment Scale (ASAS), the Physician Global Aesthetic Improvement Scale (PGAIS), the Subject Global Aesthetic Improvement Scale (SGAIS), and an acne scar quality of life questionnaire (QOLIS).

Results: At the end of Period 1, 68.2% of subjects were PGAIS responders and 79.6% were SGAIS responders. At the end of Period 2, ASAS scores of the PMMA group showed significantly the PMMA group continued for an additional 12 weeks. At the end of Period 2 the mean PGAIS change in the PMMA group was statistically larger than in the no-treatment group ($p = 0.0147$) and the PMMA improvement was maintained for an additional 12 weeks. The mean SGAIS change in the PMMA group was larger than in the no-treatment group at the end of Period 1 ($p = 0.2806$). Responses to quality-of-life questions indicated that the greater improvement compared to the no-treatment group ($p = 0.0136$) and improvement in combination treatment provided a greater change than microneedling alone. Treatment-related adverse events due to PMMA were not observed.

Conclusion: Although microneedling alone improved acne scars, injecting PMMA as well provided significant therapeutic benefit that persisted for at least 24 weeks without adverse events.

CLINICAL APPLICATIONS – CUTANEOUS: *COMPLICATIONS, LEGAL ISSUES*

CAUSES OF INJURY AND LITIGATION IN CUTANEOUS LASER SURGERY: AN UPDATE

Saami Khalifian, H. Ray Jalian, Mathew M. Avram

Massachusetts General Hospital, Boston, MA; Rebecca Fitzgerald Dermatology, Los Angeles, CA

Background: Previous work from our group identified increasing injuries and claims related to cutaneous laser surgery from 1985 to mid-2012, particularly when performed by nonphysician operators. Here, we identify and update the incidence of injury and medical professional liability claims from cutaneous laser surgery since June 2012.

Study Design/Materials and Method: Cases were searched for using an online national database to identify public legal documents related to laser injury and litigation. Additionally, data was gathered on the frequency and nature of cases, type of procedure performed, clinical injury and setting, year of litigation, certification of provider and operator, and cause of legal action.
Results: From June 2012 to Sept 2017 we identified 63 total cases related to injury secondary to cutaneous laser surgery. The incidence of litigation related to laser surgery shows an increasing trend since 1985, with peak occurrence in 2012. Laser hair removal was the most common litigated procedure. Non Physician operators accounted for a substantial subset of these cases, with their physician supervisors named as defendants, despite not performing the procedure. Plastic surgery was the specialty most frequently litigated against. Of the preventable causes of action, the most common was failure to obtain an informed consent and negligence. Of the 19 cases with public decisions, 9 (47.4%) resulted in decisions in favor of the plaintiff. The mean indemnity payment was $494,311.
Conclusion: Claims related to cutaneous laser surgery are steadily increasing, as are the average indemnity payments, which exceed the previously reported average across all medical specialties. Non Physicians account for most cases of injury and litigation. This data is consistent with prior work from our group which evaluated similar data from 1985 to mid-2012. Non Physicians performing these procedures will be held to a standard of care corresponding to an individual with appropriate training; thus, physicians are ultimately responsible for the actions of their Non Physician agents.

FDA MAUDE DATA ON COMPLICATIONS WITH NON-INVASIVE BODY CONTOURING DEVICES

Adam J. Wulkan, Mathew M. Avram
Massachusetts General Hospital, Boston, MA

Background: The FDA mandates that manufacturers and device operators disclose medical device reports (MDRs) to monitor suspected injuries and device malfunctions by submitting information to The Manufacturer and User Facility Device Experience (MAUDE) database. Given the rapid growth in the non-invasive fat reduction market, it is essential physicians be aware of associated adverse events.
Study Design/Materials and Method: Using the MAUDE electronic database on the FDA website, we performed a comprehensive search of reported complications of non-invasive fat reduction and cellulite reduction devices from January 2014 to October 1, 2017 at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfmaude/search.cfm. MDRs felt to be a medically possible adverse event were included.
Results: Our search yielded 76 MDRs, a majority submitted by patients. MDRs felt to be a medically possible adverse event are listed. 26 MDRs reported on cryolipolysis, included newly diagnosed or exacerbation of prior umbilical hernia (10), neuropathy (5), paradoxical fat hyperplasia (4), blisters (3), rash (1), flu-like symptoms (1), gastroenteritis (1), prolapsed bladder/uterus/rectum (1). Of the 11 MDRs for radiofrequency, 10 reported burns/blisters and one fire. 17 MDRs were reported for 1060 nm laser lipolysis, including burn/blister (5), nodules (4), pain (3), cellulitis/abscess (2), excessive swelling (1), neuropathy (1) and vomiting (1). All MDRs associated with focused-pulsed ultrasound reported blister/burns (4), one on a non-FDA approved body site and another after combination RF/IR treatment for cellulite reduction. There were 8 MDRs reported with 1440 nm laser cellulite reduction, including burn (5), unacceptable cosmesis (2), faulty power supply (1). There were 13 MDRs reported on high-frequency ultrasound, including burn/blister (9), subcutaneous nodules (2), excoriation (1). Vacuum-assisted subcision was associated with 5 MDRs including seroma/hematoma (1), laceration (1), festooning/unacceptable cosmesis (2), and nodules (1).
Conclusion: It can be safely presumed that most physicians do not report adverse events to the FDA; however, the FDA MAUDE database remains the largest repository of adverse event reported for non-invasive body contouring, an area that has grown tremendously over the last few years. Furthermore, several MDRs are likely unrelated and/or unsubstantiated. Additionally, number of MDRs for any given device must be correlated to the number of procedures performed. The MAUDE database allows a glimpse into potential adverse events that can occur. It is our hope that physician awareness of this database and the adverse events it reports will help improve patient safety.

CLINICAL APPLICATIONS – CUTANEOUS: *FAT REMOVAL*

A CUSTOMIZED APPROACH FOR ARM FAT REDUCTION USING CRYOLIPOLYSIS

Jason K. Rivers, Marcie Ulmer, Brianne Vestvik, Sherisse Santos
Pacific Derm, Vancouver, BC, Canada

Background: Cryolipolysis of the arms has been demonstrated to be an effective treatment for non-invasive reduction of subcutaneous fat. This study evaluated the safety and efficacy of the concurrent use of a new commercially-available small applicator in conjunction with an existing medium sized applicator for the customized treatment of arm fat in a single treatment session.
Study Design/Materials and Method: Bilateral arms of 15 eligible subjects were simultaneously treated using one or two vacuum applicators with flat contours. Either a medium or small cryolipolysis applicator with an oblong cup-shaped cooling surface was selected to treat upper arm fat. The shape of the fat bulge in each subject's arm was assessed and up to two treatment cycles (−11°C for 35 minutes each) were delivered to each arm in one session, based upon investigator discretion. Throughout the procedure and at the completion of each treatment cycle, investigators assessed the subject's level of comfort, as well as sensory and motor nerve effects. Post-treatment manual massage was performed and clinical assessments of each treatment site were recorded. Adverse events were recorded to monitor procedural safety. Baseline and 12 weeks post-treatment photographs and ultrasounds measurements were taken to assess efficacy. Subject questionnaires were administered to evaluate satisfaction.
Results: Fifteen female subjects (mean age of 51.1, mean BMI of 26.8) completed the study. Of the 15 subjects, seven were treated with one cycle using the medium applicator, two were treated with one cycle using the small applicator, two were treated with two cycles using the medium applicator, and four were treated with two cycles using the small applicator. Ultrasound imaging revealed statistically significant fat layer reduction of 2.5 mm (SD ± 2.4 mm, 95% CI 1.6–3.3). Subject surveys administered 12 weeks post-treatment demonstrated 87% satisfaction with the arm cryolipolysis procedure. A panel of blinded, independent physicians correctly identified 82% of the before and after photos. Clinical assessments found adverse events were mild and included erythema and mild swelling that

resolved without intervention. Mild treatment area numbness was reported by 73% of subjects at the 4-week interim visit and fully resolved at the 12-week visit.
Conclusion: This study documents the first reported customized approach for assessment and treatment of arm fat using a small or medium cup applicator with varied applicator placement. By incorporating one or two treatment cycles per arm in a single session, the issue of variable fat distribution in people's arms can be addressed. This approach was shown to be a safe and effective way to reduce unwanted arm fat with high patient satisfaction.

A NON-INVASIVE CRYOLIPOLYSIS DEVICE VERSUS A HIGH INTENSITY FOCUSED ULTRASOUND DEVICE FOR FAT REDUCTION: A RANDOMIZED CLINICAL TRIAL

Farhaad R. Riyaz, Amelia Geisler, Alexandra Weil, Emily Poon, Murad Alam
Northwestern Memorial Hospital, Chicago, IL

Background: In recent years, non-invasive methods have been developed to reduce subcutaneous fat such as cryolipolysis (freezing of fat cells) and high intensity focused ultrasound (HIFU; mechanical cavitation or thermal destruction of fat cells). To date, there has been a paucity of studies comparing the effectiveness of these new non-invasive fat reduction techniques. The purpose of this study was to compare the effectiveness of a series of treatments of cryolipolysis and ultrasound for reduction of subcutaneous fat of the flanks.
Study Design/Materials and Method: This was a split-body, parallel-group, randomized clinical trial. Healthy, adult female participants with a BMI of 18 to 30 and moderate fat in the abdomen and flanks were enrolled. Participants were randomized to receive 3 treatments, every 4 weeks, of cryolipolysis on one flank and HIFU on the contralateral side. Follow-up visits were conducted at 12 weeks. The primary outcome was thickness of the subcutaneous fat layer as measured by diagnostic ultrasound. Secondary outcomes were pain, patient-reported improvement, and total circumference.
Results: 12 female participants completed the study and their data were analyzed. At 12-weeks follow-up, both cryolipolysis and HIFU treatments reduced fat in the flanks ($p = 0.0007$ and $p = 0.0341$, respectively) compared to baseline, as measured by diagnostic ultrasound. There was significantly less fat in the flanks on the sides treated with cryolipolysis compared to HIFU ($p = 0.007$). Participants reported significantly more pain with HIFU compared to cryolipolysis ($p = 0.0002$). Although participants-reported improvement in the appearance of their flanks was significant for both treatments ($p < 0.0001$), there was no significant difference in improvement scores between the two treatments. Total circumference did not change throughout the study.
Conclusion: Both cryolipolysis and HIFU are effective at reducing subcutaneous fat in the flank region, with cryolipolysis being less painful and more effective at reducing fat.

CHANGES IN 3-D ULTRASOUND MEASUREMENTS IN FAT FOLLOWING A 1060 nm NON-INVASIVE DIODE LASER

Lawrence S. Bass, Robert L. Bard, Bruce E. Katz, Roy G. Geronemus
Bass Plastic Surgery, New York, NY; Bard Cancer Center, New York, NY; Mount Sinai Hospital, New York, NY; Laser and Skin Surgery Center of New York, New York, NY

Background: Previous studies have shown the use of a 2-D ultrasound system is a valid method to measure fat thickness in human subjects. In this study we examine the results of using a non-invasive 1060 nm diode laser for fat reduction of the submental area using a 3-D ultrasound system with a board certified radiologist.
Study Design/Materials and Method: An ultrasound system with a linear 3-D transducer RSP-16-D was validated and used to capture a 3-D ultrasound image of the submental area. For the validation of 3-D ultrasound measurements, 3 subjects who did not receive treatment were measured on three separate days all within one week. A total of 55 subjects were enrolled to observe the changes in submental fat thickness after up to 2 treatments with a 1060 nm diode laser. Ultrasound measurements were recorded at baseline and 12 weeks post final treatment.
Results: During the validation process the maximum difference for one subject between any two days was 5% while the minimum was 0%. The average difference in measurements between any two days ranged from −1% to −3%. The standard deviations of the measurements were 3%. Among the 55 subjects who were treated, fat thickness across all patients had an average reduction of 1.785 mm (±1.173) and 15.2% (±8.7%) when comparing the baseline measurement to the 12 week follow up. A majority of subjects (51%, 28/55) received 23% average (range of 18–35%) reduction in fat thickness in ultrasound measurements. Statistically significant changes were seen when comparing the difference in fat thickness measurement at baseline and follow up ($p < 0.01$).
Conclusion: Measurements taken with the 3-D ultrasound system were shown to be consistent and repeatable with 5% or less error. Ultrasound measurements have shown a statistically significant change in fat thickness measurements of the submental area after treatment with a 1060 nm non-invasive diode laser.

IMPACT OF EXPOSURE TIME AND POST TREATMENT MASSAGE ON SUBCUTANEOUS FAT THICKNESS REDUCTION USING A MONOPOLAR RADIOFREQUENCY DEVICE

Ashish C. Bhatia, Ginger M. Pocock, Amogh Kothare
DuPage Medical Group, Naperville, IL; Cutera Inc., Brisbane, CA

Background: Subcutaneous adipolysis has been achieved by hyperthermic treatments using Radiofrequency (RF) technology. The efficacy of hyperthermic treatments depend on two factors: temperature of the target anatomy and exposure time. Additionally, previous clinical work has demonstrated improved efficacy of fat reduction by incorporating massage post hypothermic treatment. In this study, we evaluate the impact of varying treatment times on the efficacy of subcutaneous fat reduction using a monopolar RF device.
Study Design/Materials and Method: Twenty subjects, ages 24–65 years with BMI ≥ 20 and ≤ 30, were enrolled in a four cohort contralateral study. Each cohort received a single treatment to the flank and abdominal region using a 40 cm^2 hand piece. First two cohorts compared contralateral treatments using 10 minute exposure to the 20 minute (Cohort 1) and 30 minute (Cohort 2) exposures. Cohort 3 replicated Cohort 2 and incorporated massage post treatment. Cohort 4 compared a 20 minute exposure without massage to the contralateral side incorporating massage post treatment. All cohorts maintained the skin temperatures between 44–45C. Assessments included digital photographs and ultrasound measurements of all treated areas to evaluate fat thickness reduction at baseline, 8, and 12 weeks post treatment.

Results: Subjects who received 10 minute treatment time demonstrated the greatest reduction in fat thickness (24% reduction) at the 3 month follow-up when compared to all other treatment cohorts. Massage had statistically insignificant effect on the clinical efficacy. No unexpected adverse events were recorded as a result of these treatments.
Conclusion: Fat reduction did not correlate with longer hyperthermic exposures at the 3 month follow-up. Massage was not found to be any more effective in reducing fat loss.

NON-INVASIVE SUBCUTANEOUS FAT REDUCTION USING A SMALL CONTOURED CUP CRYOLIPOLYSIS APPLICATOR IN ASIAN SUBJECTS

Samantha Y. Shek, Chi K. Yeung, Henry H.L. Chan
The University of Hong Kong, Hong Kong SAR, China

Background: Subjects with a smaller body frame may be ideal candidates for cryolipolysis given an applicator designed to cool their smaller proportions effectively. The new small contoured cup cryolipolysis applicator creates suction at the bottom of the hand piece leading to a better draw volume and less discomfort. This study explored this applicator for non-invasive fat reduction in the abdomen and flanks of Asian subjects.
Study Design/Materials and Method: Abdomens and flanks of eligible Asian subjects were treated with a small contoured cup cryolipolysis applicator. The number of areas each subject received at each session was determined by the investigator. Each subject underwent two treatment sessions approximately six weeks apart and were followed until twelve weeks after their final treatment. Any adverse effects were documented. Efficacy was assessed via photographic review of baseline versus 12-week post-final treatment photos by three blinded independent physician reviewers, caliper and circumference measurements, as well as subject satisfaction questionnaires.
Results: Twenty-four subjects were enrolled and completed treatment and follow-up. Subjects mostly experienced mild to moderate erythema and oedema immediately after treatment, however all side effects resolved by the 12-week post-final treatment visit. No device- or procedure-related adverse events were reported. Six subjects completed 12-week post-final treatment and from their Subject Satisfaction Questionnaire, 100% of subjects were satisfied with their treatment and results, and 83% would recommend the procedure to a friend. Mean fat layer and circumferential were 0.95 mm reduction and 0.98 cm increased at 12-weeks post-final treatment, respectively.
Conclusion: The small contoured cup applicator is satisfactory in reducing subcutaneous fat in the abdomen and flank in this Asian population. This study demonstrated the safe use of this applicator for small pockets of fat, and provided a good fit for patient populations that may otherwise not have been candidates for the procedure due to the smaller size of the fat bulges.

SIMULTANEOUS BILATERAL CRYOLIPOLYSIS USING A CUP APPLICATOR FOR NON-INVASIVE FAT REDUCTION IN THE ENLARGED MALE BREAST

Girish S. Munavalli
Wake Forest University, Charlotte, NC

Background: A previous study using a parallel cooling plate cryolipolysis applicator was the first published study showing non-invasive fat reduction for pseudogynecomastia. While the procedure was deemed to be safe and effective, topical anesthetic around the areola/nipple complex was needed to reduce treatment discomfort from the applicator vacuum force. The objective of the current study is to evaluate the safety, efficacy, and tolerability of a contoured cooling cup cryolipolysis applicator for non-surgical treatment of pseudogynecomastia
Study Design/Materials and Method: Enrollment consisted of 12 males with pseudogynecomastia. Subjects received simultaneous bilateral treatment consisting of a 35-minute cryolipolysis cycle, followed by a two minute massage, and a second 35-minute cycle with 50% treatment area overlap in a single treatment visit. At 6-week follow-up, subjects received a second treatment with up to two cycles per side. Safety was evaluated by monitoring side effects and adverse events. Efficacy was assessed 6 weeks after second treatment via ultrasound, clinical photographs, and subject surveys.
Results: Of the 12 enrolled, 11 subjects completed the study. Ultrasound analysis found mean fat layer reduction of 5.1 mm (32.3% reduction). Three blinded, independent reviewers correctly identified 97% of baseline photographs. Surveys revealed 100% of subjects were satisfied, 91% reported visible fat reduction, and 100% would recommend cryolipolysis treatment to a friend. Side effects included mild discomfort during treatment and transient paresthesia and tenderness.
Conclusion: This study demonstrated the contoured cooling cup applicator provides rapid, safe, effective, and well-tolerated non-surgical treatment of pseudogynecomastia. The novel applicator was more tolerable than the first-generation applicator and topical anesthetic was not required to manage comfort when treating this area.

TREATMENT OF AXILLARY HYPERHIDROSIS USING A NON-INVASIVE 1060 nm DIODE LASER

Bruce E. Katz
Juva Skin & Laser Center, New York, NY

Background: Axillary hyperhidrosis is estimated to affect 1.4% of the U.S. population. Newer treatments such as microwave technology, botulinum toxin injections and lasers have emerged as effective methods. In this pilot study we examine the use of a non-invasive 1060 nm diode laser for the treatment of axillary hyperhidrosis.
Study Design/Materials and Method: 10 subjects were enrolled in a pilot study and received two treatments with a 1060 nm laser on the axillary area. Starch iodine tests were performed and 2-D photography was taken before and after treatment. Subjects were asked to rate their condition on the Hyperhidrosis Disease Severity Scale (HDSS) and satisfaction with treatment. Two blinded dermatologist evaluators graded the reduction in sweat production on photos of starch iodine tests. Retrospective analysis was performed on all subjects to further assess the safety and efficacy of the treatment.
Results: The blinded evaluators rated 75% reduction in sweat production in 80% of the subjects. The remaining subjects rated 50% improvement. Average HDSS score was 3.8 (+/−0.4) before treatment and 2.2 (+/− 0.4) 6 months post 2 treatments. All of the subjects indicated that they responded to treatment and reported that their results were maintained at follow up. 80% of the subjects were satisfied with treatment. Retrospective analysis showed that all adverse events were transient and resolved without intervention. Common side effects were treatment pain, oedema and erythema which resolved within a few weeks.
Conclusion: The use of a non-invasive 1060 nm diode laser is an effective and safe method for the treatment of axillary hyperhidrosis.

CLINICAL APPLICATIONS – CUTANEOUS: *LASER-ASSISTED DRUG DELIVERY*

LASER-ASSISTED DELIVERY OF TOPICAL POLY-L-LACTIC ACID IN THE TREATMENT OF UPPER LIP RHYTIDES: A PROSPECTIVE, RATER-BLINDED TRIAL

Omer Ibrahim, Sarah Ionta, Joaninha P. Depina, Kathleen G. Petrell, Kenneth A. Arndt, Jeffrey S. Dover

SkinCare Physicians, Chestnut Hill, MA

Background: Perioral rhytides are a common complaint among dermatologic patients and can be notoriously stubborn and resistant to therapy. We aimed to assess the efficacy and safety of fractional ablative laser-assisted delivery of topical poly-l-lactic acid (PLLA) suspension in the treatment of upper cutaneous lip rhytides.

Study Design/Materials and Method: Prospective, single-arm, rater-blinded trial. Ten subjects with moderate to severe upper lip rhytides underwent three bimonthly treatments of low-density fractional carbon dioxide laser followed by topical application of PLLA suspension. Wrinkle severity before and after treatment was analyzed using computer-generated analyses. Blinded raters and subjects assessed improvement of wrinkles after treatment using the Global Aesthetic Improvement Scale (GAIS) (scores ranging from −3 to 3).

Results: After 3 treatments, the severity of upper lip wrinkles decreased by an average of 47% ($p < 0.05$) as calculated by computer-generated image analyses. Blinded raters as well as subjects rated wrinkles as much improved after 3 treatments (score of 2 on the GAIS). No unanticipated adverse events were noted.

Conclusion: Laser-assisted delivery of PLLA is a safe and effective treatment for upper lip rhytides.

CLINICAL APPLICATIONS – CUTANEOUS: *PHOTOAGING AND PICOSECOND*

FRACTIONAL 1064 nm AND 532 nm PICOSECOND-DOMAIN LASER TREATMENT OF FACIAL PHOTODAMAGE

Eric F. Bernstein, Kevin Schomacker, Jayant Bhawalkar

Main Line Center for Laser Surgery, Ardmore, PA; Syneron-Candela, Corp., Wayland, MA

Background: A novel fractionated laser delivering 532 nm and 1064 nm picosecond-domain pulses was used to treat facial photodamage, and assessed for safety and effectiveness.

Study Design/Materials and Method: A total of 27 subjects were treated with a novel 1064 nm or 532 nm, Nd:YAG, picosecond-domain laser fitted with a novel handpiece incorporating holographic beam splitters to create small fractionated microbeams. One group of subjects was treated with 1064 nm ($n = 17$), and the other with 532 nm ($n = 10$). Improvement in the appearance of photodamage was evaluated by three blinded assessors comparing parallel polarized photographs taken before and 3 months following the final treatment. A total of 5 or 4 monthly treatments were administered for the 1064 nm and 532 nm wavelengths, respectively.

Results: A total of 24 of the 27 enrolled subjects completed all study visits. Baseline wrinkle scores averaged 5.1 ± 1.8 and 6.6 ± 1.7 for the 1064 nm and 532 nm cohorts, respectively, and decreased to 3.9 ± 1.7 and 5.6 ± 1.7 following treatment. A total of 72 image assessments were available for this analysis (24 image pairs, 3 assessors). The treated image was correctly identified in 52 of the 72 image assessments (one sided $p = 0.0001$). For the images that were correctly identified, the mean percent improvement was 27% for 532 nm treated subjects, and 25% for the 1064 nm group. Side effects were limited to mild-moderate discomfort during treatment, and mild-moderate erythema, oedema, purpura and petechiae.

Conclusion: This novel fractionated picosecond-domain, 1064 nm and 532 nm Nd:YAG laser safely and effectively improved facial photodamage with little to no downtime.

PICOSECOND 1064 nm LASER FOR TREATMENT OF PIGMENTATION AND PHOTOAGING IN DARK SKIN TYPES

Neil S. Sadick

Weill Cornell College, New York City, NY

Background: Facial skin rejuvenation and pigmentation in darker skin types has traditionally been addressed with Q-switched 1064 nanosecond lasers. New generation picosecond 1064 nm lasers have emerged to compete these traditional treatments by targeting epidermal and dermal pigmentation, as well as superficial textural irregularities associated with photoaging. As picosecond lasers rely on a photo-mechanical effect as opposed to a purely thermal response they can achieve improved clearance of melanin but also significantly heightens the wound healing response for rejuvenation without causes side effects such as post-inflammatory hyperpigmentation. In this study the safety and efficacy of picosecond 1064 nm laser was evaluated in dark-skinned patients with pigmentation and photoaging.

Study Design/Materials and Method: 20 patients (ages 22–65) with Fitzpatrick skin type III–VI with melasma, moderate photoaging and lentigos participated in this prospective case series. All patients received a total of 2 treatments with a 1064 nm picosecond laser ($0.6\,J/cm^2$, 5 Hz, 8 mm, 4 passes) at 4 week intervals. Follow assessments were done at 12 weeks following the last treatment by blinded investigator using the Global Aesthetic Improvement Scale (GAIS) scale. Patient satisfaction was evaluated using a self-assessment questionnaire.

Results: All treatments were rated comfortable and no adverse effects were reported. 87% of patients rated the results as satisfactory for melasma improvement and very satisfactory for improvement of photoaging. Blinded investigator assessment rated the improvement of melasma as improved and the degree of photoaging as very improvement.

Conclusion: This prospective case series demonstrates that new generation picosecond lasers are safe and effective skin rejuvenation and reduction of pigmentation in patient of darker skin types.

SAFETY AND EFFICACY OF A PICOSECOND 755 nm WAVELENGTH ALEXANDRITE LASER WITH FOCUS LENS ARRAY FOR THE TREATMENT OF NECK LAXITY

Hana Jeon, Daniel A. Belkin, Roy G. Geronemus

Laser & Skin Surgery Center of New York, NY

Background: Non-invasive treatment of neck laxity has been a growing area of interest in the field of dermatologic surgery, and many technologies have been proposed to offer nonsurgical alternatives for patients. The purpose of the study was to evaluate the safety and efficacy of the treatment of skin laxity on the neck using a picosecond 755 nm laser with focus lens array.

Study Design/Materials and Method: A prospective, single center study using a picosecond 755 nm laser with focus lens array (Cynosure) was performed to evaluate the efficacy of treating neck laxity. Twenty-five patients were enrolled. The treatment parameters were 0.71 J/cm^2, 6 mm spot size, pulse width of 750 picoseconds, and 10 Hz. Each patient received five treatments to the neck every two to four weeks (average of three weeks). Follow-up visits were done at one month and three months following the last treatment. Digital photographs were taken at each visit. Patient and physician satisfaction scores as well as Global Aesthetic Improvement Scales (GAIS) were assessed.
Results: Twenty-four patients completed the study (21 females, 3 males). The patients' Fitzpatrick skin types ranged from I to IV (I 8%, II 72%, III 16%, and IV 4%). The age ranged from 39 to 65 (average 58). The number of total pulses were 5042 on average (range 5006 to 5612). The majority of patients did not require anesthesia (84%), and the average pain score during the treatment was 4.7 on a 0 to 10 scale. Cool air was used for 84% of the treatments to provide comfort. On average, mild redness following the treatment lasted for less than a day (0.6 days, range 0 to 5 days), and mild pain lasted for less than a day (0.1 days, range 0 to 2 days). Patients did not experience any swelling, crusting, bruising, infection, scarring, or dyspigmentation. On average, physicians were satisfied with 42% of the treatments, with 13.5% being extremely satisfactory, and were neutral with 40% of the treatments. Physician GAIS noted improvement for 57% of the treatments. Thirty-one percent of the patients were satisfied, 18% extremely satisfied, and 38% were neutral. Seventy-four percent of patients reported that they were likely to recommend the treatment to their friends and family members.
Conclusion: A picosecond 755 nm laser with focus lens array can serve as a safe nonsurgical treatment option for neck rejuvenation in Fitzpatrick skin type I–III patients, especially for those who seek treatments with minimal to no downtime. Further studies are needed to identify the clinical characteristics of neck laxity that would most benefit from this treatment.

CLINICAL APPLICATIONS – CUTANEOUS: *PIGMENT*

A COMPARATIVE STUDY WITH A PICOSECOND ALEXANDRITE WITH A DIFFRACTIVE LENS ARRAY OR A FLAT OPTIC IN DARK ASIAN PATIENTS IN A SUNNY ENVIRONMENT

Niwat Polnikorn, Emil A. Tanghetti
Chaophya Hospital, Bangkok, Thailand; Center for Dermatology and Laser Surgery, Sacramento, CA

Background: Melasma is especially challenging to treat in dark, Asian patients living in a sunny environment. The use of a Q-switched Nd:YAG in a procedure referred to as laser toning has delivered inconsistent results and reported cases of hypopigmentation in this patient population. The picosecond Alexandrite laser with the diffractive lens array can selectively treat and diminish areas of hyperpigmentation in the epidermis with minimal thermal damage to the skin by creating an area of laser induced optical breakdown. Our objective is to describe a technique with the fractional optic on the 755 nm picosecond Alexandrite laser and compare it to a widely used treatment method with the flat optic on this device.
Study Design/Materials and Method: In Bangkok the picosecond Alexandrite laser at 755 nm was evaluated. Group 1 (24 patients) were treated with the flat optic at 1.2–2.0 Joules/cm^2, 1 *pass*. Group 2 (24 patients) were treated with the diffractive array at 0.41 Joules/cm^2 with the 8 mm optic. All patients were at 1 hertz with no overlap. Both groups were treated at two week intervals for 6 treatments. Melasma Area Severity Index (MASI) scores were assessed pretreatment and at 1,3, and 6 months after the final treatment. Topical 4% alpha arbutin was applied for maintenance in both groups.
Results: In Group 1, 10 subjects dropped out due to post treatment hyperpigmentation and downtime. The remaining 14 subjects had an increase in MASI scores at 1 month. At 6 months, MASI scores improved <50% in the majority of cases. Group 2, a smaller increase in MASI scores was observed at one month. At six months, MASI scores were excellent in 7 cases (29.17%), and good in 6 cases (25%). 54.17% of cases had good-excellent results. 3 cases in Group 1 developed macular hypopigmentation with no occurrence in group 2.
Conclusion: This picosecond alexandrite laser with a diffractive optic and slow measured approach can be used to successfully treat a significant number of dark skinned Asian patients with melasma in a sunny climate.

A PROSPECTIVE, SPLIT-FACE, RANDOMIZED STUDY COMPARING PICOSECOND LASER TO Q-SWITCHED LASER FOR TREATMENT OF EPIDERMAL AND DERMAL PIGMENTED LESIONS IN ASIANS

Woraphong Manuskiatti, Chanida Ungaksornpairote, Rungsima Wanitphakdeedecha
Faculty of Medicine Siriraj Hospital, Bangkok, Bangkoknoi, Thailand

Background: Picosecond laser operates with pulse duration of the sub-nanosecond range. Its efficacy in removal of unwanted tattoo pigments is superior compared to Q-switched lasers, as a higher peak of temperature is generated as well as a better thermal confinement. Nevertheless, the performance of picosecond laser to outperform Q-switched laser in treating pigmented lesions has yet to be evaluated. The objective of this study was to compare the efficacy and safety of picosecond and Q-switched lasers in treating solar lentigines and acquired bilateral nevus of Ota-like macules (ABNOMs) in Asians.
Study Design/Materials and Method: Eight subjects presented with solar lentigines and six subjects with ABNOMs were enrolled. Each of the 14 subjects were randomly treated with a picosecond laser on one side of the face and a Q-switched laser on the other. Subjects with the lentigines received only one treatment, while the ABNOMs subjects were treated five times every 12 weeks. Evaluation on subjective assessment on the degree of pigment clearance, and adverse effects (clinical evaluation by two blinded dermatologists) were obtained at week 0, 4, 12, and 24 after the final treatment.
Results: There was a statistically significant difference in clinical improvement between the two laser systems at week 4 ($p = 0.034$), week 12 ($p = 0.039$), and week 24 ($p = 0.027$) follow-ups. At the 6 months follow up, 85.7% of picosecond and 57.2% of Q-switched laser sites showed a 50% improvement. There was no significant difference in the incidence of side effects and healing time. However, picosecond laser was significantly associated with a lower treatment discomfort ($p = 0.05$) compared with Q-switched laser.
Conclusion: Picosecond laser appears to be more effective and better tolerated than Q-switched laser for the treatment of epidermal and dermal pigmented lesions in Asians.

ASSESSMENT OF COMBINED FRACTIONAL CO_2 AND TRANEXAMIC ACID IN MELASMA TREATMENT

Shereen O. Helal, Dalia Abdelhalim, Mohamed Abdelhady, Amira El-Barbary
Cairo University, Cairo, Egypt

Background: Melasma continues to be a difficult to treat disease. Role of fractional CO_2 in treatment is controversial. Addition of Tranexamic acid appears to be helpful in treatment. The objective is to assess the efficacy of low power fractional CO_2 laser alone in the treatment of Melasma, and to compare it with a combination of low power fractional CO_2 laser with Tranexamic acid used both topically and intralesionally.
Study Design/Materials and Method: A double blind comparative study included a total of thirty female patients with bilateral, symmetrical Melasma. They were randomly divided into two study groups. In both groups, the right side of the face was subjected to low power fractional CO_2 laser only. On the left side of the face, in addition to being subjected to low power fractional CO_2, in group A, it was followed by topical application of Tranexamic acid solution, while in group B, it was subjected to intralesional microinjection of Tranexamic acid prior to low power fractional CO_2 laser. Sessions were received every 4–6 weeks for 5 consecutive sessions. Assessment was done using the MASI score, melanin index (MI), and erythema index (EI) before sessions and two weeks after the last session.
Results: After treatment, significant reduction in the MASI score was noted in both right and left (groups A&B) sides (p value 0.007, $<$0.001, 0.016 respectively). MI was significantly reduced in both the right side and group B (p value $<$0.001, 0.003 respectively), while the EI had significant improvement only on the right side (p value 0.023). On comparing the degree of improvement between the different treatment modalities, the degree of improvement regarding the MASI score was better on right side of the face (1.75 ± 2.15), followed by group A (1.70 ± 2.62), then group B (1.45 ± 1.89). Regarding MI, the degree of improvement was better in group B (27.40 ± 26.62), followed by the right side of the face ($25.75 \pm 32 \pm 63$), then group A (18.69 ± 33.43). EI showed better degree of improvement on the right side of the face (5.21 ± 11.73), followed by group A (18.69), then group B (3.60 ± 7.85). As regards degree of improvement in MASI score, MI, EI, no statistical significance was noted between the right side of the face and the left side (groups A&B) (p value 0.933, 0.715, 0.728) respectively. Minimal complications occurred in the form of mild pain following the sessions. None of the patients suffered prolonged erythema, ulceration, infection or scarring.
Conclusion: Low power fractional CO_2 laser is an effective, safe treatment for Melasma. Addition of Tranexamic acid to fractional CO_2, either topically or intralesionally proved to be an effective adjuvant therapy.

CLINICAL EVALUATION OF A NOVEL PICOSECOND 670 nm WAVELENGTH FOR THE TREATMENT OF BENIGN PIGMENTED LESIONS

Omar A. Ibrahimi, Ginger Lau
Connecticut Skin Institute, Stamford, CT

Background: Treatment of benign pigmented lesions is a commonly requested procedure in aesthetic dermatology. Selective photothermolysis of benign pigmented lesions can be accompanied by many side effects, including hyper- and hypo-pigmentation. Here, we evaluate the clinical efficacy of a novel 670 nm wavelength in the treatment of benign pigmented lesions.
Study Design/Materials and Method: A pilot prospective, self-controlled, single center study of benign pigmented lesion removal was designed consisting of 10 subjects. The study consisted of up to ten treatments at 6–8 week intervals using a picosecond/nanosecond laser platform consisting of a novel 670 nm wavelength and a follow up visit 90 days after the last laser treatment. Clinical efficacy of lesion removal was determined by blinded physician assessment of improvement at 12 weeks post final treatment using digital pictures.
Results: 10 subjects enrolled into the study treatment protocol. Treatment efficacy data is awaiting completion of the follow up portion of the study but demonstrates the utility of this novel 670 nm wavelength in the treatment of benign pigmented lesions.
Conclusion: A novel picosecond/nanosecond 670 nm wavelength is safe and effective for the treatment of benign pigmented lesions and provides a new therapeutic option for patients with unwanted benign pigmented lesions.

ENHANCED TREATMENT OF MELASMA WITH COMBINED SURFACE AND DIFFRACTIVE LENS OPTICS

Margaret A. Weiss, Robert A. Weiss
Maryland Laser, Skin & Vein Institute, Baltimore, MD

Background: Melasma is very difficult to treat. Results with picosecond lasers is encouraging but may not be successful longer term. This study was performed to investigate whether surface and subsurface optics would enhance results.
Study Design/Materials and Method: In this study 24 patients with significant melasma were treated with a both a diffractive lens array lens ($0.71\,J/cm^2$) and a surface optic ($2\,J/cm^2$) for a series of 4 treatments using a 755 nm picosecond laser. Treatments were performed at 4 week intervals.
Results: Results indicated significant reduction of pigment intensity as measured by UV photography in 18/24 subjects. Moderate reduction was observed in 4 patients and 2 had no improvement. Images were judged by blinded ratings at 6 months. No adverse events were recorded.
Conclusion: Dual passes with both diffractive lens and surface optics show significant effect on melasma. This dual pass treatment shows promise for improving results on a difficult clinical presentation. More clinical observation at one year is currently being pursued.

FIRST CLINICAL USE OF NON-THERMAL NANO-PULSE STIMULATION TO ELIMINATE SEBORRHEIC KERATOSIS LESIONS

Thomas E. Rohrer, Omer Ibrahim, Nicole Lee, Adele Haimovic, Kathleen G. Petrell, Joaninha P. Depina, Sarah Ionta, Pamela Buckman, Edward A. Ebbers
SkinCare Physicians, Chestnut Hill, MA; Pulse Biosciences, Inc., Hayward, CA

Background: Nano-pulse stimulation (NPS) is the non-thermal, localized delivery of a timed series of low energy, high voltage nanosecond pulses that can trigger programmed cell death as evidenced by nucleolysis of exposed cells. The cell-specific effect in tissue takes place in the shallow depth of epidermis in the treated area, and spares the non-cellular elements of the dermis. This study represents the first time seborrheic keratosis (SK) lesions have been treated with this type of device.

Study Design/Materials and Method: 13 adult subjects were required to have at least 4 off-face lesions clinically diagnosed as SK lesions and within study criteria for size. A local anesthetic was injected prior to treatment. Three lesions were treated in a single session, and one lesion was left untreated as a control. Subjects returned five times over an 106-day period for physician assessment of the degree of clearing of SK lesions and the cosmetic appearance of treated tissue. Visits occurred at day 7, 30, 60, 90 and 106 post-treatment. At the final 106-day visit, subjects rated their satisfaction with treatment. Blinded, independent reviewers will rate the degree of clearing of SK lesions based on photographs taken at each visit.
Results: Preliminary efficacy measurements for subjects having completed the 60-day visit showed 77% of treated SK lesions as cleared or mostly cleared. At the time of this submission, no adverse events have been reported. The most frequently reported residual skin condition was mild to moderate hyperpigmentation at 60-days and no reports of hypopigmentation. The 90-day and 106-day follow-up visits and data analysis will be completed prior to the presentation.
Conclusion: NPS demonstrates potential as a new treatment for SK lesions. Larger studies and longer follow-ups are needed to validate these promising early results.

LASER TREATMENT FOR POST INFLAMMATORY HYPERPIGMENTATION IN PATIENTS WITH DARKER SKIN TYPES: A RETROSPECTIVE PHOTOGRAPHIC REVIEW

Yoon-Soo Cindy Bae, Samantha Rettig, Elliot T. Weiss, Leonard J. Bernstein, Roy G. Geronemus
Laser & Skin Surgery Center New York, New York, NY; Cornell University, New York, NY

Background: Post inflammatory hyperpigmentation (PIH) can be difficult to treat especially in patients with darker skin types as darker skin carries increased epidermal melanin content. Various treatments available to improve the appearance of PIH may incite further pigmentation thus making treatment of these skin conditions extremely difficult and frustrating. The purpose of this study was to perform a retrospective chart and photographic review to evaluate the efficacy and safety profile of a low energy low density non-ablative fractional diode 1927 nm wavelength laser (Solta, Hayward, CA) treatment for post inflammatory hyperpigmentation in patients with Fitzpatrick skin types IV–VI.
Study Design/Materials and Method: A retrospective evaluation of 61 patients with post inflammatory hyperpigmentation treated with a 1927 nm laser was conducted at a single center. Inclusion criteria required at least 2 treatment sessions so that before and after treatment photographs would be available for comparison and study purposes. Two blinded physician-evaluators using a visual analog scale for percentage of pigmentary clearance in standard photographs assessed treatment efficacy. Adverse events were noted throughout the patient charts.
Results: The majority of patients receiving 1927 nm laser treatments improved on average of 44%, for rater 1 and 43%, for rater 2, with a pearson's correlation coefficient of 0.59. According to a paired t-test, no significant differences between rater 1 and rater 2 were observed ($p = 0.64$). No side effects were observed in the patients treated with this laser modality.
Conclusion: The low energy low density non-ablative fractional diode 1927 nm wavelength laser is a safe and effective approach for improving post inflammatory hyperpigmentation in patients with darker skin types.

PROSPECTIVE STUDY OF REMOVING SOLAR LENTIGINES USING PICOSECOND 532 nm Nd:YAG LASER FOR ASIAN PATIENTS

Kei Negishi, Hirotaka Akita, Saori Tobita
Institute of Geriatrics, Tokyo Women's Medical University, Shibuya, Tokyo, Japan; Banbuntane Houtokukai Hospital, Fujita Health University, Nagoya, Aichi, Japan; Clinic Sure Ginza, Chuo, Tokyo, Japan

Background: Picosecond lasers have been used for removing benign pigmented lesions, but there have been few reports for darker skin patients. The objective of this study was to evaluate the safety and efficacy of a picosecond Nd:YAG laser for removing solar lentigines in Asians.
Study Design/Materials and Method: A prospective, single site, IRB approved study. Fifty solar lentigines in 26 subjects (24 female, 2 male), skin type III or IV, mean age 57.2, received up to two treatments using 532 nm, 370 picoseconds, 4.5 mm spot size, $0.54+/-0.09\,J/cm^2$. Clinical endpoint was >75% clearance evaluated by the treating physician. Results were evaluated at one and three-month post-treatment. Two blinded assessors evaluated improvement, and melanin index (MI) measured by colorimeter was analyzed for objective evaluation. Subject self-assessments were also obtained. Biopsies were obtained in two cases to compare histological differences between QS laser and picosecond laser.
Results: Forty-three lesions (86%) needed one treatment and seven lesions (14%) needed two treatments to achieve >75% clearance. Adverse effects were seen in five cases: post-inflammatory hyperpigmentation in two cases (7.7%), hypopigmentation in two cases (7.7%) and prolonged erythema in one case (3.8%). Assessors evaluated >86% of lesions into the highest grade (>76% clearance) on a 5-grade percentage scale at both one and three-month follow-up. Improvement of relative MI (MI in lesion minus MI in normal area) was $79.17+/-32.03\%$ and $77.27+/-22.28\%$ at one and three-month follow-up, respectively ($p < 0.001$). Subject self-evaluation showed >80% of lesions in the highest grade in 5-grade improvement scale. Pain-score was $3.36+/-0.78$ on a 0–10 scale. Histology showed epidermal vacuolar formation in both QS and picosecond laser treatment, however, size was much smaller in picosecond laser.
Conclusion: Picosecond 532 nm Nd:YAG laser treatment safely removed solar lentigines in Asian patients with limited epidermal damage.

RETROSPECTIVE STUDY OF PICOSECOND ALEXANDRITE 755 nm LASER FOR NEVUS OF OTA TREATMENT IN CHINESE

Tong Lin, Lin Peng, Yin Yang, Yiping Ge
Institute of Dermatology, Chinese Academy of Medical Sciences, Nanjing, Jiangsu, China

Background: Nevus of Ota is a benign dermal pigment condition which presents as brownish–gray patches on the faces. Although the use of Q-switched lasers for nevus of Ota in Chinese patients have shown good clinical results. However, multiple sessions with a long treatment interval are still required. Recently, picosecond lasers were introduced in the treatment of nevus of Ota. The objective of this study was to evaluate the efficacy and safety of picosecond alexandrite laser for nevus of Ota in Chinese.
Study Design/Materials and Method: 29 patients with nevus of Ota from October 2015 to December 2016 were

included in this study. All subjects were Fitzpatrick skin type III or IV. A picosecond alexandrite laser was used to treat all patients. The treatment parameters were: pulse duration of 750 ps, spot sizes of 2–4 mm and fluence of 1.95–6.37 J/cm^2. Clinical photographs were taken at baseline and prior to every treatment session. Images were evaluated independently by two dermatologists. A five-point grading scale, complete clearance 95–100%, excellent 75–94%, good 50–74%, fair 25–49%, minimal 0–24%, was used for the evaluation of lesion clearance.
Results: Patients underwent a range from 1 to 5 treatment sessions. Among those patients who received only one treatment, more than 75% clearance was achieved in 8 patients. 50%–74% clearance in 5 patients with one treatment, and less than 50% in 16 patients with one treatments. After a single treatment, cure rate depending on colour fading and area reduction was 20.7% while the effective rate was 44.8%. Two patients with hyperpigmentation after treatment were observed.
Conclusion: The picosecond alexandrite laser showed significant improvement in Chinese nevus of Ota patients over 3 or fewer treatments with minimal adverse events.

SUCCESSFUL AND SAFE RESULTS IN THE TREATMENT OF COMBINED LESIONS OF MELASMA WITH BLEMISHES USING COMBINATION TREATMENT OF Q-SWITCHED 1064 nm Nd:YAG LASER AND 755 nm PICOSECOND LASER

Joonhong Park
Maymorning Skin Clinic, Sungnam, Kyunggi, Korea

Background: In Asian patients with pigment disorders, there are much more patients who have a complicated mix of melasma and blemishes than melasma or blemishes alone. In these cases, a successful and safe treatment is very difficult to achieve because iatrogenic PIH is more likely to occur than separate blemishes or melasma cases. While treating pigmented disorders in Asian patients, safety is the most important factor. This study demonstrated safe and effective treatment for combined pigmentary disorder with melasma and blemishes with combination treatment with a Q-switched 1064 nm Nd:YAG laser and a 755 nm picosecond laser.
Study Design/Materials and Method: This was a retrospective chart review of 154 patients of melasma with blemishes which included lentigines(127), freckles(11), ABNOM(11) and others with unclear diagnosis(5). Patients were treated with Q-switched 1064 nm Nd:YAG laser for melasma first, immediately followed by 755 nm picosecond laser. The treatment parameters for Q-switched 1064 nm Nd:YAG laser was 3.3–3.9 J/cm^2 fluence, 7.0–7.5 mm of spot size, PTP mode with the endpoint of mild erythema and for the 755 nm picosecond laser was 1.59 J/cm^2, 4 mm spot size with one or two passes over the blemish lesions. The Q-switched 1064 nm Nd:YAG laser treatment was performed weekly for ten to twenty sessions, and the 755 nm picosecond laser was performed every two weeks.
Results: Assessment revealed excellent response (76–100% improvement) in 64% of patients and good to excellent response (51–100% improvement) in 87% of patients. Eight out of 154 patients had PIH.
Conclusion: The combination treatment of Q-switched 1064 nm Nd:YAG laser and 755 nm picosecond laser for combined lesions of melasma and blemishes in Asian skin of type IV through V is safe and effective.

THE USE OF 1064 nm Nd:YAG PICOSECOND LASER FOR THE TREATMENT OF HYPERPIGMENTED SCARS: A CASE REPORT SERIES

Amir Koren, Roni Niv, Ofir Artzi
Tel Aviv Sourasky Medical Center, Tel Aviv, Gush Dan, Israel; Epilogic Laser Center, Jerusalem, Tel Aviv, Gush Dan, Israel

Background: The severity of scars is usually evaluated according to a number of characteristics, one of which is hyperpigmentation. The picosecond laser technology has been in clinical use for the treatment of tattoos and a variety of pigmentary disorders since 2012.
Study Design/Materials and Method: Nine healthy patients with typically hyperpigmented scars, underwent 3–5 1064 nm Nd:YAG picosecond (Candela, 1.7–2.5 mj/μbeam) laser treatments at 3–6-week intervals. Subjective assessment of the response to treatment was made by both the patient and the treating dermatologist on a visual analog scale (VAS). The before and 4 months after treatment photographs were compared for treatment efficacy by two non-involved dermatologists using the global assessment scale (GAS). In addition, the patients rates their satisfaction and tolerance. A mexameter was used to quantitatively evaluate the melanin content of the scar before and after the laser treatments.
Results: 8/9 treating dermatologists and 6/9 patients reported moderate–to-good responses on the VAS. The 2 dermatologists' scoring on the GAS demonstrated a good-to-excellent response. The average satisfaction rate was high (2.77), and the average patient tolerance level was good (3.66). The melanin content of the scar decreased by an average of 50.27% compared to pretreatment values.
Conclusion: The fractionated non-ablative picosecond Nd:YAG laser was shown to be effective for the treatment of the hyperpigmented component of scars.

THE USE OF A NOVEL, LOCALIZED, CONTROLLED SKIN COOLING SYSTEM FOR REDUCTION OF EPIDERMAL PIGMENTATION

Henry H.L. Chan, Dieter Manstein, Mathew M. Avram, Vincent Zuo, Joseph Ting, R. Rox Anderson
Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, Blossom Innovations Boston, MA

Background: Lasers have been used for the treatment of epidermal pigmentation but can be associated with complications such as PIH. Melanocytes are known to be very susceptible to cryoinjury, however such injury is typically reported after application for cryosurgery and results in long lasting depigmentation and side effects. The dosimetry and role of controlled skin cooling on epidermal pigmentation deserves further investigation. The objective is to evaluate the safety and effectiveness of a novel system when used for localized controlled cooling of the epidermal layer to reduce pigmentation in the absence of adverse events.
Study Design/Materials and Method: 2 female Sinclair Yucatan pigs were treated on day 0 on the left and right flank, using the controlled cooling system with a flat cooling surface on 57 treatment sites and 8 positive control sites and with a cooling surface system with a microstructured cooling surface at a total of 72 treatment sites and 8 positive control sites, leaving 6–7 untreated negative control sites per animal. The temperature and the exposure time of the cooling surface were varied. Digital photography was used to document the

treatment sites during the study with images used for photographic review scoring. Biopsy samples for histopathology were taken of untreated sites before the treatment procedure and from treatment sites immediately following treatment (Day 0), then from untreated and treatment sites monthly thereafter. Clinical observations were performed at least once daily for 14 days, then weekly thereafter

Results: Microscopic evaluation revealed that localized epidermal cooling applied by device treatment was associated with favorable tissue responses, and with decreases in melanocyte/pigmentation parameters. Compared with the continuous surface hand piece, treatment with the indented surface hand piece was associated with more consistent outcome. Specific exposure conditions were determined (cooling range $-3\,^{\circ}C$ to $-9\,^{\circ}C$ with exposure time 15–60 seconds) that would consistently and safely elicited a response and that showed at least a mild reduction in pigmentation (score of 3 or lower) based on the photographic review. There was absence of any notable side effects.

Conclusion: We have demonstrated the use of a novel controlled cooling system in a pig model that reduce epidermal pigmentation without any marked epidermal damage and side effects.

TREATMENT OF BENIGN PIGMENTED LESIONS USING A NOVEL PRE-BETA DERMAL COOLING SYSTEM: CLINICAL UTILITY AND PATIENT EXPERIENCES AT A UNIVERSITY-BASED PRACTICE

Aria Vazirnia, Arisa E. Ortiz
University of California San Diego, La Jolla, CA

Background: Cryotherapy is a painful and antiquated method for treating benign pigmented lesions, frequently resulting in blistering and dyschromia. Light-based devices are more selective therapeutic strategies, yet may still result in post-inflammatory pigmentary alterations, especially in darker Fitzpatrick skin types (FST). We present our clinical experience with a novel FDA-cleared dermal cooling system for the treatment of benign pigmented lesions. This system applies controlled, localized cooling to the epidermis to temporarily reduce melanin production.

Study Design/Materials and Method: We report early clinical experience using the pre-beta Dermal Cooling System for benign pigmented lesions. Initial participation is limited to FST I-III; with patients receiving up to 3 treatments. Treatment parameters were determined based on lesion location, thickness, and FST. The cooling handpiece was directly applied to an interface to protect the epidermis, and masking was utilized to confine the cooling to discrete lesions. The cooling treatment consisted of between 107 and 166 kJ/m^2 of extracted heat from the tissue, immediately followed by a controlled rewarm. Clinical photography was taken at baseline, and at 1 and 2-month follow-up visits.

Results: Clinical experience is ongoing. Controlled dermal cooling demonstrated improvement in pigmentation with minimal downtime. Treatments were well-tolerated and associated with essentially no pain. Thinner lesions responded better than thicker lesions, such as thick seborrheic keratoses. Notably, the dermal cooling system was very effective at treating macular seborrheic keratoses on the extremities which have been previously resistant to various treatment modalities. Acceptable pigment improvement required 1–3 treatments. Anticipated side effects included temporary treatment site redness, dryness, itching, and transient darkening. No long-term hyper- or hypo-pigmentation has resulted to date.

Conclusion: This novel, essentially painless dermal cooling system demonstrates promise in the treatment of benign pigmented lesions. Additional clinical experience is needed to optimize treatment parameters and determine which lesions respond best to dermal cooling.

CLINICAL APPLICATIONS – CUTANEOUS: *REJUVENATION*

ASSESSING CONSUMER KNOWLEDGE, OPINIONS AND APPROACH TO OBTAINING COSMETIC PROCEDURES

Adam J. Wulkan, Cortney Donaldson, Louis Scafuri, Mathew M. Avram
Massachusetts General Hospital, Boston, MA; Zalea, Irvine, CA

Background: In 2016, there were an estimated 15.9 million cosmetic procedures performed in the United States by a variety of healthcare providers ranging from dermatologists, plastic surgeons, facial plastic surgeons, nurses, aestheticians, dentists, *etc.* Little is known regarding how consumers approach these procedures and how they choose their providers. This study attempts to assess how consumers research, self-educate, and choose cosmetic procedures and providers.

Study Design/Materials and Method: Survey recipients were sourced randomly from an electronic survey system, which was also used to conduct a twenty-question survey. Respondents qualified by having obtained or by having considered a cosmetic procedure.

Results: 931 individuals were polled, of which 323 (35%) met inclusion criteria, with 84 (9%) of individuals having had a cosmetic procedure and 239 (26%) considering one. Most individuals relied on their physician (67%) family/friends (57%), and Google search (51%) to obtain relevant information. Of individuals who had a cosmetic consultation, 42.5% had a procedure performed. The most popular cosmetic procedures individuals considered include laser hair removal (28%), laser/light therapy (25%), abdominoplasty (25%), injectables (24%) and non-invasive fat reduction (24%). When asked whether they were medical doctors, plastic surgeons led the way with 89%, followed by dermatologists (82%), dentists (52%), aesthetician (20%) and nurses (11%). 82% of individuals checked physicians credentials prior to treatment. Regarding expertise, facial plastic surgeons were most respected with 63% considering them the best, followed by plastic surgeons (55%), and dermatologists (30%).

Conclusion: This online survey provides insight into consumer preferences and knowledge regarding cosmetic procedures and providers. Furthermore, it identifies an education gap regarding which providers are medical doctors. With a growing number of non-physician aesthetic providers, education appears to be essential to allow the public to make well-informed decisions regarding their cosmetic procedures.

LOOKING YOUNGER TEN YEARS LATER: IMPROVEMENT IN AGE ASSESSMENT AS JUDGED TODAY COMPARED TO BASELINE PHOTOGRAPHY TEN YEARS AGO, THE LONG TERM BENEFIT OF AESTHETIC MEDICINE

Lauren E. Boudreaux, Douglas J. Key
Good Samaritan Regional Medical Center, Salem, OR; Key Laser Institute, Portland, OR

Background: Our patients in noninvasive aesthetics hope to gain an improvement in their appearance of age as judged relative to their actual age. Studies that confirm an

improvement in judged age as relative to actual age as a benefit of non-invasive aesthetic treatment over the longer time span of one decade are lacking.
Study Design/Materials and Method: For inclusion patients must have a) been with the practice for approximately ten years, b) underwent various aesthetic procedures over that time period with associated before-and-after Canfield photography, and c) had their age at the time of photography recorded. Patients (n = 72, 59 women, age range 25–83 years) were selected, with associated photographs before treatment and after their last intervention, covering an average period of 10.71 ± 1.32 years. Photographs were examined by 11 non-expert graders not side-by-side, but rather in random order, along with duplicate photographs of 50 untreated individuals. Reviewers guessed and recorded perceived age of patients in each photograph. The difference between the actual age at the time of each photograph and the estimated age was determined and compared with that of the after picture to derive a numerical value. These values were averaged from among the 11 graders for each patient photo set, followed by statistical testing for confidence interval (CI), which was used to assess patient response to treatment.
Results: Of 72 patients, approximately 93% (n = 67) were graded as responders (27 low, 17 medium, and 23 high responders), meaning that average perceived age improved, as per the non-expert graders.
Conclusion: Statistical analysis of before and after photographs over time may serve to validate the long term efficacy of cosmetic treatments and provide insight for aesthetic providers into their practice.

CLINICAL APPLICATIONS – CUTANEOUS: *SCARS*

LASER TREATMENT IN EARLY WOUND HEALING TO PROMOTE PHYSIOLOGICAL SKIN REMODELING – A RANDOMIZED CONTROLLED TRIAL

Katrine E. Karmisholt, Christina Banzhaf, Uwe Paasch, Alexander Nast, Merete Haedersdal
Bispebjerg Hospital, Copenhagen, Denmark; University of Leipzig, Leipzig, Germany; Charitè Universitetsmedizin Berlin, Berlin, Germany

Background: Today, scarring is inevitable following surgical procedures. Laser treatment for mature scars is established but complete remission to normal skin is uncommon. Within recent years, a preventative approach has been adopted by applying laser during early wound healing to minimize scar formation, but a study investigating multiple non-ablative-fractional-laser (NAFL) treatments in the early wound healing phases is lacking. The aim of this study is to explore the clinical effect of three NAFL-exposures applied respectively in 1) inflammation-, 2) proliferation- and 3) remodeling-phase of wound healing to reduce scar formation.
Study Design/Materials and Method: In a randomized, controlled, spilt-scar trial, 1540 nm NAFL-treatments were applied to surgical wounds $\geq$2.5 cm; immediately before surgery, at suture removal and one month after surgery versus no laser. NAFL-exposures were applied with deep and superficial energy depositions (range 40–50 mJ/microbeam). Primary endpoint comprised blinded on-site physician assessment of scars by Patient-Observer-Scar-Assessment-Scale (POSAS, range 1 = normal skin to 10 = worst imaginable scar) at 1 and 3 months follow-up. Secondary endpoints were skin reflectance measurements, blinded Vancouver Scar Scale (VSS) evaluation, blinded photo evaluation and patients' satisfaction at 3 months follow-up.
Results: A total of 32 patients were included. At 1 month follow-up especially pliability and relief of treated scars were improved compared to controls, but scars were still affected with erythema from NAFL-treatments (data currently for n = 24). Preliminary results at 3 months follow-up showed improvement of POSAS-total and VSS on NAFL-treated scar side compared to controls. Data from all participants, clinical photos as well as visualization by reflectance confocal microscopy of treated and untreated scar sides will be presented.
Conclusion: This is the first study investigating the clinical effect of three NAFL-treatments all applied in the early wound healing phases starting as early as immediately before surgery. Blinded clinical evaluation is promising towards improvement on NAFL-treated scars compared to untreated scars.

PULSED DYE LASER TREATMENT PRIOR TO SURGICAL EXCISION

Catherine M. DiGiorgio, Kachiu C. Lee, Martin Purschke, Mathew M. Avram, R. Rox Anderson
Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA; Brown University, Providence, RI

Background: The 595 nm pulsed-dye laser (PDL) can be used after cutaneous injury to improve the appearance of scars. Based on the theory of selective photothermolysis, PDL targets oxyhemoglobin – the predominant type of hemoglobin present in erythematous and hypertrophic scars. The objective of this study is to determine whether use of the PDL prior to surgical excision can prevent scar formation.
Study Design/Materials and Method: This was a prospective, randomized split-scar pilot study using the 595 nm PDL immediately prior to surgical excision. Experimental halves of surgical sites were exposed to PDL at varying fluences and pulse durations to achieve an endpoint of transient purpura. Control halves received PDL at minimal fluences or were left untreated. Subjects returned at weeks 2, 4, 6, and 8–12 months post-treatment for digital photography, patient scar assessment and blinded observer scar assessment scales.
Results: Ten subjects were included in the study. Overall, there was no clinically significant difference in the treatment site compared to the control in the patient scar assessment scale at week 6 ($p = 0.8798$) and month 8–12 ($p = 0.8773$) or blinded observer scar assessment scale at week 6 ($p = 0.9378$) and month 8–12 ($p = 0.3341$). Correspondingly, no significant difference in the 10 clinical endpoints measured by the patient scar assessment and blinded observer scar assessment scales was observed. Although not significant, overall scores from blinded observers rated the treatment site worse in appearance, while subjects rated the treatment site as
better compared to control. No significant adverse events occurred, though three subjects experienced surgical wound dehiscence.
Conclusion: PDL treatment prior to surgical excision does not improve the clinical appearance of surgical scars and the results of our study suggest there is no benefit for the use of PDL prior to surgical excision.

REPORTING THE PHENOMENON OF "PERSISTENT PIXEL STAMPING MARKS", FOLLOWING FRACTIONAL CARBON DIOXIDE LASER REJUVENATION OF SCARS

Samia M. Esmat, Mona Rabie Abdelhalim, Marwa M. Kamel, Amany Z. El-Ramly, Kholoud Helmy, Heba Gawdat, Soheir Esmat
Cairo University, Cairo, Egypt

Background: Fractional CO_2 laser rejuvenation of scars offers a high safety profile. The laser marks usually disappear clinically within one week. The authors observed that this is not always the rule and that some scars might show persistence of the laser marks on the skin for more than four weeks. We named it "Persistent Pixel Stamping Marks".

Study Design/Materials and Method: Cases were randomly recruited from patients attending for scar rejuvenation at the Laser Unit, Faculty Of Medicine, Cairo University. during the period from January to April 2017. Those included 182 patients with different types of scars. The development of "Pixel Stamping Marks" was observed by three investigators. Those who developed the phenomenon where subjected to clinical and Dermoscopic examination, imaging by optical coherence tomography (OCT) as well as 4 mm punch biopsy. A biopsy was taken from twenty randomly selected cases before and one month after the first sessions whether they developed the marks or not and served as controls All sections were stained with H&E, Masson's Trichrome, Masson Fontana, and Orcein stains and were followed up for at least six months.

Results: "Persistent Pixel Stamping Marks" developed in 18 cases (9.8%), sixteen of which were post burn hypertrophic scars. The development of pixel stamping marks is significantly related to the darker skin type ($p = 0.05$) and darker scar colour ($p = 0.001$) and the longer its age ($p = 0.008$).Laser parameters did not affect the incidence of the marks. Histopathological findings included the presence of single or combined findings of characteristic holes in the stratum corneum and superficial dermis, thick collagen bundles perpendicular to the skin surface with loss of elastic tissue, focal interface changes, and triangular focus of fibroblastic proliferation. Follow-up for six month showed that the marks disappeared in ten and persisted in eight patients. Persistence of the marks was significantly associated with using longer dwell time and lower densities.

Conclusion: "Persistent Pixel Stamping Marks" can be considered a form of miniature scars at the sites of the microscopic thermal zones or a sign of their delayed healing. It occurs following fractional CO_2 laser resurfacing, especially of hyper-pigmented, long standing burn scars in darker skin individuals. Using shorter dwell times and higher densities makes them disappear faster.

CLINICAL APPLICATIONS – CUTANEOUS: *SKIN CANCER*

1064 nm Nd:YAG LASER FOR THE TREATMENT OF BASAL CELL CARCINOMA: DETERMINING LONG-TERM EFFICACY

Arisa E. Ortiz, Mathew M. Avram
University of California – San Diego, San Diego, CA; Massachusetts General Hospital, Boston, MA

Background: Basal cell carcinoma (BCC) is the most common type of skin cancer, yet current nonsurgical options require a prolonged treatment course and have high rates of recurrence. The need for a more effective and efficient nonsurgical alternative has led to recent investigations of 1064 nm Nd:YAG lasers for the treatment of BCC. Our recent multi-center study showed 90% histologic clearance at 1 month with one 1064 nm Nd:YAG laser treatment. However, long-term clearance rates have yet to be determined. The objective of this study is to monitor the long-term clearance rates of BCC with one 1064 nm Nd:YAG laser treatment.

Study Design/Materials and Method: This is an ongoing, prospective, multi-center study evaluating the long-term efficacy of the 1064 nm Nd:YAG laser for the treatment of BCC. Patients seeking treatment for BCC in clinic that are poor surgical candidates or who do not wish to undergo surgery are recruited. Subjects receive one treatment with the 1064 nm Nd:YAG laser. Patients are monitored for recurrence every 6 months at their standard in-office skin exams. Standardized photographs and adverse assessments are taken pre- and post-laser treatment and at follow-up visits.

Results: No recurrences have been clinically identified to date. Treatments are well tolerated with locally injected anesthesia. Expected immediate side effects include oedema and erythema. The majority of lesions heal with barely perceptible scarring with only a few treatments that resulted in minimal scarring that was considered acceptable to the patient. Treatments were covered under the CPT malignant destruction codes.

Conclusion: The 1064 nm long-pulsed Nd:YAG laser is a new addition to our armamentarium for treatment of BCC and is proving to be a reasonable alternative for patients that do not wish to undergo surgical treatments or those that are poor surgical candidates. Research is ongoing to determine long-term clearance rates.

A SINGLE-CENTER CLINICAL STUDY APPLIES MELANOMA IMAGING BIOMARKERS TO STANDARD AND HYPERSPECTRAL DERMOSCOPY FOR EARLY MELANOMA DETECTION

Anna-Marie Hosking, Kenneth G. Linden, Kristen M. Kelly, James Jakowatz, Maki Yamamoto, Janellen Smith, Dorothy Chang, Samantha Lish, Sung Lee, Manuel Valdebran, Faezeh Talebi-Liasi, Dorota Korta, Daniel S. Gareau
University of California, Irvine, CA; UC Irvine Health Chao Family Comprehensive Cancer Center, Orange, CA; Rockefeller University, Laboratory of Investigative Dermatology, New York, NY

Background: Early detection of melanoma is essential to decrease associated morbidity and mortality. Screening classically involves dermoscopy to identify suspicious lesions as benign nevi, atypical nevi, or cancerous growths, with subsequent biopsy and histological examination. However, with current methods, a considerable number of unnecessary biopsies are performed. With the aid of image processing and artificial intelligence algorithms, we have the potential to generate standardized melanoma imaging biomarkers (MIBs). MIBs are characteristics extracted from quantitative analysis of dermoscopy images that correlate with melanoma pathology. MIBs have been shown to be spectrally dependent in Red/Green/Blue (RGB) colour channels, suggesting hyperspectral imaging may further enhance diagnostic power.

Study Design/Materials and Method: After obtaining informed consent, pigmented lesions of 20 patients from UC

Irvine were imaged with both standard and hyperspectral dermatoscopes prior to removal and histopathological analysis of suspicious lesions. Each image underwent automated computer analysis, leading to a set of MIBs. Machine learning was applied to combine the MIBs with histological diagnoses, creating a predictive algorithm to identify each pigmented lesion as melanoma or nevus.
Results: The sensitivity and specificity results for melanoma diagnosis in preliminary analysis of 20 lesions were specified by the standard receiver/operator characteristic curve. In addition, hyperspectral imaging biomarkers contained a more robust set of diagnostic information than imaging biomarkers derived from standard dermoscopy. Additional cases will be accrued and analyzed in the final results.
Conclusion: Imaging biomarkers show promise to noninvasively screen melanoma and guide biopsy. With this novel, non-invasive methodology for evaluating pigmented lesions, dermatologists can harness computational power to aid in standardized evaluation. Over time, and with the analysis of more lesions, the computer can gain additional expertise in this area. Continuing work aims to improve the power of the study as well as further investigate the additional diagnostic value of hyperspectral image analysis over RGB image analysis.

CARBON DIOXIDE LASER ABLATION OF BASAL CELL CARCINOMA GUIDED BY REFLECTANCE CONFOCAL MICROSCOPY

Anthony M. Rossi, Brian P. Hibler, Kishwer S. Nehal, Miguel Cordova, Oriol Yelamos
Memorial Sloan Kettering Cancer Center, New York, NY; Hospital Clínic, Universitat de Barcelona, Barcelona, Spain

Background: Basal cell carcinoma (BCC) is the most common skin cancer and its incidence is rising. Laser ablation is a safe, rapid treatment for BCC, but lack of histological clearance is a limitation. Reflectance confocal microscopy (RCM) allows *in vivo* visualization of skin with cellular-level resolution. We wish to evaluate the effectiveness of RCM-guided carbon dioxide (CO_2) laser ablation to treat early BCCs.
Study Design/Materials and Method: Superficial or early nodular BCC were included. RCM imaging was performed pre-ablation to define lateral and deep margins and define the laser parameters. After ablation, RCM was performed in the wound to identify residual BCC. If residual tumour was observed, additional laser passes were performed. Patients were evaluated clinically, dermoscopically, and with RCM at 1, 6 and 12 months, and with continued clinical surveillance thereafter.
Results: Twenty BCCs in 7 patients (4 male, 3 female) were treated. Median age was 55 years (range 29–74). Eighteen (90%) were on the limbs and trunk, two (10%) on the head and neck. Median lesion diameter was 7 millimeters (range 3–12 mm). The medial laser passes was 3 (range 2 to 8). At 1 month, an erythematous depression was observed in all cases which improved by 6 months. With average follow-up 17 months (range 4–29), no recurrence has been detected clinically, dermoscopically or by RCM. All sites show good cosmetic outcome, and RCM shows dermal features of scar.
Conclusion: Our results suggest that CO_2 laser ablation guided by RCM is an effective, minimally-invasive treatment for superficial and early nodular BCC with good cosmetic outcome. Larger studies with long-term follow-up are warranted to confirm our findings.

CLINICAL APPLICATIONS – CUTANEOUS: *SKIN TIGHTENING*

A PROSPECTIVE, RANDOMIZED, SINGLE-CENTER, EVALUATOR-BLINDED CLINICAL TRIAL EVALUATING THE SAFETY, EFFICACY, AND PATIENT SATISFACTION OF INJECTABLE CALCIUM HYDROXYLAPATITE IN COMBINATION WITH MICRO-FOCUSED ULTRASOUND FOR REJUVENATION OF THE AGING CHEST

Marwan Alhaddad, Monica Boen, Sabrina G. Fabi, Douglas Wu, Mitchel P. Goldman
Cosmetic laser Dermatology, San Diego, CA

Background: Cosmetic rejuvenation of the décolletage has shifted to favor the utilization of multiple cosmetic modalities at the same session to target the multiple aspects of the aging skin, such as combining dermal fillers with various energy devices. Calcium hydroxylapatite (CH-P; Merz Aesthetics) functions by inducing an immediate soft tissue augmentation effect as well as a prolonged stimulation of neocollagenesis for a more durable result. Microfocused ultrasound with visualization (MFU-V; Ulthera, Inc., Mesa, AZ) technology delivers energy to specific soft tissue layers beneath the epidermis. Focal 1 mm^3 microthermal lesions are produced at approximately 65 °C which induces contraction of collagen fibers and a subsequent tissue remodeling response. The ultimate effect is tightening and lifting of the skin. In light of the above evidence, combination therapy with CH-P and MFU-V is hypothesized to synergistically target the foundations of cutaneous revitalization of the décolletage: volume restoration, neocollagenesis, and tissue contraction.
Study Design/Materials and Method: This was a prospective, randomized, evaluator-blinded, single-center, triple- armed comparison clinical trial that enrolled 60 subjects with each arm containing 20 subjects. The first arm was treated with CH-P to the chest alone. The second arm was treated with MFU-V to the chest alone. The third arm was treated with a combination of both MFU-V and CH-P to the chest at the same session.
Results: Subjects treated with CH-P alone and MFU-V alone both showed significant decreases in dynamic and static chest wrinkles over time, while subjects treated with a combination of CH-P and MFU-V did not ($p = < 0.01$, <0.01, and NCS, respectively, Single Factor ANOVA, Microsoft Excel). No major adverse events reported with any of the three treatment arms.
Conclusion: Treatment with CH-P alone or MFU-V alone, showed statistically significant improvement in chest wrinkles, with no statistically significant differences between the two arms in investigator- assessed and patient-assessed measures. Interestingly, when the two modalities are combined in the same treatment session, our data failed to show any statistically significant changes at any time point in the appearance of wrinkles on the décolletage.

BIPOLAR RADIOFREQUENCY NASAL TIP AND EARLOBE REFINEMENT

Macrene R. Alexiades
Dermatology & Laser Surgery Center of New York, New York, NY

Background: Nasal tip and earlobe refinement are plastic surgical procedures requiring excision of the fibrofatty portion of the nasal tip and skin of lobule, respectively. Radio frequency

(RF) bipolar energy is a well-established modality for non-invasive skin reshaping on face and body areas. RF-induced heating of the nose or earlobe may cause partial denaturation of fibrofatty tissue and skin, respectively, that result in tissue contracture and skin tightening through dermal remodeling.
Study Design/Materials and Method: Ten subjects with enlarged or bulbous nasal tips and/or earlobes were enrolled. Each received 3 monthly treatments with bipolar RF at 30–45 J/cm^2 until a uniform skin surface temperature of 40–42 °C was attained. Subjects were followed at 1, 3 and 6 months.
Results: Reductions in the diameter and length of the nasal tip and ear lobule were observed in all subjects. Mean diameter reductions were measured at 1.1 mm for nasal tip and 2.8 mm for earlobes following 3 monthly treatments at 6 month follow up. Results were statistically significant.
Conclusion: Bipolar RF-mediated refinement is a safe and effective non-surgical procedure on the nose and earlobe.

EFFECTIVENESS OF FRACTIONAL 1550 nm LASER VERSUS FOCUSED ULTRASOUND FOR THE TREATMENT OF PERIORBITAL RHYTIDS: A RANDOMIZED CLINICAL TRIAL

Sreya Talasila, Amelia Geisler, Kapila Paghdal, Wanjarus Roonpisuthipong, Emily Poon, Murad Alam
Northwestern University, Chicago, IL

Background: One common complaint in patients with an aging face is periorbital rhytids. Minimally invasive treatments for rhytids include non-ablative fractional laser (fractional 1550 nm Er:Glass laser) and non-invasive skin tightening devices (microfocused ultrasound tightening device). However, the comparative effectiveness of such nonablative energy treatments has not been well-studied.
Study Design/Materials and Method: This is a split-face randomized clinical trial with 1:1 allocation. The purpose of this study is to assess and compare the effectiveness of fractional 1550 nm laser and focused ultrasound for the treatment of periorbital rhytids. Healthy, adult participants with Fitzpatrick phototype I–III and with lower eyelid and crow's feet rhytids at rest were enrolled. Participants were randomized to receive 2 treatments, 8 weeks apart, of fractional non-ablative laser to one side of their face, while the contralateral side received microfocused ultrasound. Follow-up visits were at 1 and 3 months after final treatment. 2 blinded dermatologist photorated improvement using a global improvement scale and a quantitative eyelid laxity measure. Adverse events were recorded at each visit.
Results: 17 participants completed the study. No adverse events were reported. Both treatments resulted in 65–70% improvement in wrinkles and there was no significant difference between the treatment modalities at the 1- and 3- month follow-up visits. Eyelid texture, laxity, and wrinkles all significantly improved from baseline at 1 month (laser: $p = 0.01$, $p = 0.001$, $p = 0.02$, respectively; ultrasound: $p = 0.003$, $p = 0.01$, $p = 0.02$, respectively) and 3 month follow-up visits (laser: $p = 0.002$, $p < 0.0001$, $p = 0.002$, respectively; ultrasound: $p = < 0.0001$, $p = < 0.0001$, $p = 0.01$, respectively), but no significant difference was observed between the treatment modalities.
Conclusion: Both fractional nonablative laser and microfocused ultrasound resulted in significant improvement of periorbital rhytids, but there was no difference in effectiveness between the two treatments. Therefore, physicians may offer both treatments as effective options for the treatment of periorbital rhytids.

IMPROVING SKIN LAXITY AND THE APPEARANCE OF LINES IN THE NECK AND DÈCOLLETAGE USING COMBINED TREATMENT WITH MICROFOCUSED ULTRASOUND AND DILUTED CALCIUM HYDROXYLAPATITE

Gabriela R. Casabona
Clínica Vida, São Paulo, Brazil

Background: Laxity and rhytides on the neck and décolletage can indicate age as reliably as the face. The objective of this study was to evaluate the combination of microfocused ultrasound with visualization (MFU-V) and diluted calcium hydroxylapatite filler (CaHA) to correct lines and wrinkles on the neck and décolletage.
Study Design/Materials and Method: A total of 47 subjects with moderate-to-severe lines on the neck and/or décolletage were retrospectively enrolled. Following application of MFU-V using 7 and 10 MHz transducers, subdermal injections of diluted CaHA (1:1 with lidocaine) were performed. Subjects were photographed at baseline and at 90 days; photos were assessed by 2 independent, blinded evaluators using the Fabi-Bolton chest wrinkle scale, Merz Aesthetics décolleté wrinkles scale, and Allergan transverse neck lines scale.
Results: A total of 29 subjects received treatment to the neck only, and 5 subjects received to the décolletage only. Thirteen subjects were treated in both areas both areas. Mean neckline score improved from 2.6 (moderate-to-severe lines) at baseline to 1.3 (mild lines) 90 days after treatment ($p < 0.001$). Décolletage scores improved from 2.6 and 3.3 (moderate-to-severe wrinkles) on the Merz Aesthetics and Fabi-Bolton scales, respectively, to 1.1 and 1.8 (mild wrinkles), respectively, after treatment ($p < 0.001$ for each scale). Both MFU-V and the CaHA injections were well tolerated, and subjects reported high levels of satisfaction.
Conclusion: The combined use of MFU-V and diluted CaHA injection (1:1) is effective for improving the appearance of lines and wrinkles of the neck and décolletage.

NEW THERAPEUTIC APPROACH OF MICRO INSULATED NEEDLE RF TO NON-SURGICAL EYEBAG TREATMENT

Gun Young Ahn, Chang-Hun Huh
Chungdam Gowoonsesang Dermatology Clinic, Seoul, Korea; Seoul National University Bundang Hospital, Seoul, Korea

Background: A lower eyelid fat bulging is one of the characteristics of cosmetic disfigurement in the aging process. Treatment modalities for eyebag have been very limited and the traditional treatment modality was only represented by surgical procedures. We performed a study which aimed to evaluate the efficacy of micro insulated needle RF system as a new modality for the non-surgical treatment by reducing lower eyelid fat volume.
Study Design/Materials and Method: This study aims to evaluate the efficacy of RF micro-insulated needle to remove lower eyelid fat bulging. Twenty-four volunteers (4 males and 20 females) with lower eyelid fat bulging were enrolled in this study. The average age of these subjects was 56 ± 6.28 years. 24 subjects with lower eyelid fat bulging were treated using micro insulated needle RF system; each subject underwent this treatment two times, four weeks apart. Two types of partially insulated needles with different lengths were used in each session. A three-dimensional photogrammetry system was used to objectively measure the change in height of the fat bulge. Investigator's global assessment for the severity of fat bulging and subject's satisfaction score were evaluated.
Results: The average height of fat bulging measured by the 3D photogrammetry was decreased significantly after 12 weeks and

maintained until 24 weeks. Investigator's global assessment score was significantly decreased after 4 weeks and further decreased after 12 weeks, which was then maintained until 24 weeks. Subject's satisfaction score also showed good results. There were no side effects, except lower lid swelling for a week.
Conclusion: Micro insulated needle RF system can be an effective and safe treatment option to treat lower eyelid fat bulging.

NON-SURGICAL MICRO-INVASIVE NEEDLE BASED BIPOLAR RADIOFREQUENCY FOR VOLUMIZING AND TIGHTENING ON FACE AND NECK

Katharina Russe-Wilflingseder, Elisabeth Russe
Plastic Reconstructive Aesthetic Surgery, Lasercenter Innsbruck, Innsbruck, Tirol, Austria; Hospital of the Barmherzige Brüder, Paracelsus Medical University Salzburg, Salzburg, Austria

Background: The demand on non-surgical skin tightening procedures for face and neck with minimal to no downtime is increasing enormously. The objective of this study is to evaluate a micro-invasive needle based bipolar radiofrequency device using real-time temperature feedback mechanism to create fractional dermal injuries in the reticular dermis to treat skin laxity and in the subdermal layer to reduce unwanted fat.
Study Design/Materials and Method: 12 patients (10 females, 2 male) between 50 and 61 years (Ø 56) received a single treatment using fractional radiofrequency (Syneron Candela) delivered through five 32g-needle electrode pairs in an angle of 25° for the reticular dermis or seven 32g-needle electrode pairs in an angle of 75° for deeper scars and fat at 67°C and 4 seconds. On average 390 adjacent insertions were applied on cheeks, perioral, jawline and neck. Treatments were performed in topical and tumescent anesthesia.
Results: Patients tolerated treatment well. Oedema and moderate bruising lasted until day 4–5 after the treatment. Our patient group showed an improvement of skin firmness, thickness, size of pores and scars. It is also noticeable the increase of volume due to the stimulation of collagen, elastin and hyaluronic acid, as well as a better definition of the jawline and creating a more youthful oval facial shape. These two signs were specially marked in patients with longer FU up to 6–12 months. Patient satisfaction was rated as 72% at 1 month follow-up and 76% at 3 months follow-up, physician rated improvement as 75% in GAIS score at 3 months follow-up. No side were seen. All patients would recommend this treatment to a friend or family member.
Conclusion: This non-surgical procedure is safe and effective to achieve a more youthful appearance of face and neck. This new device tightens face and neck by improving skin thickness and elasticity, wrinkles and scars and reduces submental unwanted fat. Downtime is minimal, especially in comparison to other procedures. Patient satisfaction and acceptance was high.

OPEN-LABEL, SINGLE-CENTER, SINGLE-TREATMENT, SAFETY AND EFFECTIVENESS EVALUATION OF PERCUTANEOUS RADIOFREQUENCY IN ACHIEVING SUBMENTAL LIFT – INTERIM DATA

Jeanine B. Downie, Barry E. DiBernardo
Mountainside and Overlook Hospital, Montclair, NJ; New Jersey Plastic Surgery, Montclair, NJ

Background: Sun damage, weight loss and natural aging are just a few of the processes that lead to skin laxity and an aged appearance. In recent years the neck and submentum have become a focus for improved techniques and with that, demands for minimally invasive treatments for lifting and tightening loose skin has increased. The objective of this study is to evaluate interim data looking at the clinical efficacy and safety of percutaneous temperature controlled radiofrequency to achieve lift and aesthetic improvement in the submentum and jawline.
Study Design/Materials and Method: 40 of a total of 70 patients with mild to moderate submental laxity and mild submental fat completed this open label, prospective, single center study. Each subject received 1 percutaneous temperature controlled radiofrequency treatment to the submental region at Day 0. Subjects were seen for follow-up visits at 30, 90, and 180 days post treatment. The primary efficacy endpoint was defined as submental lift $\geq 22\,mm^2$. Secondary endpoints included Subject and Physician Global Aesthetic Improvement Scales (SGAIS, PGAIS) assessing overall lift, Subject and Physician global satisfaction questionnaires assessing: skin appearance, skin texture (tightness, firmness, smoothness), and overall results, and a Subject questionnaire assessing likelihood to recommend the procedure. An additional exploratory endpoint assessing improvement in skin elasticity was also examined.
Results: The interim data demonstrates improvement on the GAIS in the appearance of submental lift with maximum results seen at 90 days: 92.3%/94.9%, and high satisfaction rates continued through 180 days: 85%/95% for both subjects and physicians respectively. Similar results were seen for subject and physician global satisfaction assessing: skin appearance at 90 days: 92.3%/92.3%, skin texture (tightness) at 90 days: 69.2%/89.7, (firmness) at 90 days: 61.5%/51.3%, (smoothness) at 90 days: 46.2%/41%, and overall satisfaction at 90 days: 82.1%/87.2%. At 180 days 75% of subjects said they were likely to recommend the treatment to others. The average improvement in skin elasticity was 28% at 180 days post treatment. There were no serious adverse events and no unexpected treatment related adverse events.
Conclusion: Percutaneous temperature controlled radiofrequency is safe and effective for the treatment of submental laxity, and demonstrates success in improving skin appearance, texture, and elasticity.

PATIENT SATISFACTION AND OUR EXPERIENCE WITH 436 MICROFOCUSED ULTRASOUND TREATMENTS

Yunyoung C. Chang, Jennifer Croix, Srimanasi Javvaji, Anne M. Chapas, Jennifer L. MacGregor
Union Square Laser Dermatology, New York, NY; Illinois Dermatology Institute, Skokie, IL; Center for Dermatology and Cosmetic Laser Surgery, Plano, TX

Background: Microfocused ultrasound (MFUS) is a safe and effective method for non-invasive skin tightening. Previous clinical studies demonstrate a 60–80% patient satisfaction after MFUS. We used an anonymous online platform after MFUS to assess patient satisfaction.
Study Design/Materials and Method: Patients treated with MFUS between January 2013 and 2016 were invited to complete an anonymous online survey at least 4 months post-treatment. Patients were asked to rate improvement in skin tightening as none (0%), mild (0–25%), moderate (26–50%), significant (51–75%), or dramatic (76–100%) and treatment satisfaction as disappointed, neutral, satisfied, or extremely satisfied.

Results: Between January 2013 and 2016, 245 patients received 436 MFUS treatments at our center. A total of 83 surveys were received with data. Nearly 80% of responders reported at least mild improvement, with 14.5% indicating significant improvement, 27.7% indicating moderate, 37.3% indicating mild, and 20.5% indicating none. In addition, 53.1% of responders reported being satisfied or extremely satisfied with the results. 44.6% of responders did not feel treatment results met expectations. All of these patients indicated that this was due to insufficient results while 13.5% also indicated pain and 5% cost of the treatment as factors. Notably, 50% of patients less than 40 years old were disappointed with their result, which was greater than for other ages.
Conclusion: Patient satisfaction with elective cosmetic procedures is an important indicator of success. Our current study demonstrates a high response rate, with almost 80% of responders indicating at least mild tightening with MFUS treatment. Interestingly, only 53.1% of patients reported satisfaction after treatment, a lower satisfaction rate than reported in previous non-anonymous studies and much lower than patients report in our office follow up appointments. This discrepancy may be due to gratitude bias. Anonymous surveys likely provide a more accurate assessment of patients' perceptions and will improve physician's future counseling efforts.

REDUCTION OF THE VOLUME AND WRINKLES UNDER THE EYES USING NON-ABLATIVE Er:YAG 2940 nm LASER ON THE LOWER EYELID PALPEBRAL CONJUNCTIVA

Jong Gu Kim
Cheonan Oracle Clinic, Seoul, Korea

Background: The main purpose of this study was to evaluate the safety and efficacy of laser irradiation of lower eyelid palpebral conjunctiva tissue with non-ablative 2940 nm Er:YAG for the reduction of volume and wrinkles under the eyes.
Study Design/Materials and Method: This is a case series study in which the patients seeking the reduction of volume and wrinkles below the eyes were randomly selected to be treated with novel non-ablative fractional Er:YAG 2940 nm laser. The treatment consists of four passes along the conjunctiva with 7 mm fractional spot and 250 msec pulses with 1.8 Hz and 3.0, 3.5, 4.0 and 4.5 J/cm^2.Each patient received three laser sessions with 2 weeks interval. Patient clinical outcome was evaluated by independent investigators using high resolution before and after photography. Score of improvement was rated according to five grade Likert scale. Patients were also asked about discomfort (VAS 0–10), potential adverse effects, and their general satisfaction with the treatment results. Follow-up was done after each session and at 1 month after the last session.
Results: Treatment was performed on 30 patients (4 male and 26 female), mean age 48 (range 28–68); Fitzpatrick skin type lll and IV at the Oracle Dermatology Clinic, Cheonan, Korea between September 2014 and January 2015. Investigators assessed just one patient without improvement, 16 had mild and moderate and 13 significant and excellent improvement. 87% of patients were satisfied with results. No adverse effects were observed after the treatment which was very well tolerated by all patients. The average pain evaluated by the patients was 2/10.
Conclusion: The novel non-ablative Er:YAG laser treatment using intra-eyelid technique may be a useful tool for reduction of volume and wrinkles below the eyes for Asian skin types. It showed to be efficacious and safe without downtime and adverse effects.

THE USE OF A RADIO FREQUENCY DEVICE FOR NON-INVASIVE EYEBROW LIFTING

David H. McDaniel, Paul M. Graham, Monica K. Li
McDaniel Institute of Anti Aging Research, Virginia Beach, VA; Project Skin MD, Vancouver, British Columbia, Canada

Background: Non-ablative radio frequency (RF) technology is commonly used for skin tightening and stimulation of dermal neocollagenesis. Anecdotal reports during treatment of periorbital wrinkles have suggested possible brow lifting effect. This study was designed to evaluate the efficacy and safety of using RF technology for brow lifting.
Study Design/Materials and Method: 30 subjects were recruited in a prospective IRB approved study and received 5 treatments using a 4 MHz monopolar RF device at 4 week intervals on their forehead and periorbital area. Subjects were healthy non-smoking males or females between 35 and 65 (53+/−7.5) years of age with brow laxity. 30, 120 and 180 day follow up visits were performed. A safety assessment was also performed at 1 week post treatment. Digital images were obtained and analyzed. Physician and subject satisfaction scores and adverse events were assessed.
Results: Fitzpatrick skin types treated included II(20%), III(46%), IV(29%), V(3%)and VI(3%). A 15 mm hand piece was used for the forehead and 10 mm for the eye area targeting an epidermal temperature of 39–44 °C. Average treatment time was 20 minutes. 30, 22 and 21 subjects were assessed at 30, 120 and 180 days post treatment respectively. Physician assessment of the brow indicated results were seen as early as 30 days post treatment and sustained improvement was seen at 180 days. 82% of subjects were satisfied at 120 day follow up, and the majority of the subjects were satisfied at the 180 day follow up. Improvement in wrinkles and skin texture were also seen. The most common side effects were mild to moderate oedema and erythema that lasted about a day. No serious adverse events were reported.
Conclusion: This radio frequency device produced brow lifting with improvement lasting 180 days post treatment with minimal side effects.

CLINICAL APPLICATIONS – CUTANEOUS: *TATTOO*

CLINICAL EVALUATION OF A NOVEL PICOSECOND 670 nm WAVELENGTH FOR THE TREATMENT OF MULTICOLOURED TATTOOS

Omar A. Ibrahimi, Ginger Lau
Connecticut Skin Institute, Stamford, CT

Background: Multi-coloured tattoos remain a treatment challenge in laser tattoo removal. Many commercially available systems are limited in their ability to treat the spectrum of tattoo ink colours. Here, we evaluate the clinical efficacy of a novel 670 nm wavelength in the treatment of unwanted tattoos.
Study Design/Materials and Method: A pilot prospective, self-controlled, single center study of laser tattoo removal was designed consisting of 10 subjects. The study consisted of up to ten treatments at 6–8 week intervals using a picosecond/nanosecond laser platform consisting of a novel 670 nm wavelength, along with 1064 nm and 532 nm wavelengths and a follow up visit 90 days after the last laser treatment. Tattoo removal was quantified using blinded evaluators using a commonly accepted quartile grading system for laser tattoo removal. The level of treatment-associated pain, and adverse events were additional study endpoints.

Results: 10 subjects enrolled into the study treatment protocol. Treatment efficacy data is awaiting completion of the follow up portion of the study but demonstrates the utility of this novel 670 nm wavelength in the treatment of multicoloured tattoos.
Conclusion: A novel picosecond/nanosecond 670 nm wavelength is safe and effective for the treatment of multicoloured tattoos. Traditionally treatment recalcitrant colours such as green and blue respond well to 670 nm wavelength and provide a new therapeutic option for patients with multicoloured tattoos seeking laser tattoo removal.

PERFLUORODECALIN-INFUSED PATCH IN PICOSECOND AND Q-SWITCHED LASER-ASSISTED TATTOO REMOVAL: SAFETY IN FITZPATRICK IV–VI SKIN TYPES

Selina S. Hamill, Paul M. Friedman
Dermatology & Laser Surgery Center, Houston, TX

Background: The topical transparent perfluorodecalin-infused silicone patch has been demonstrated to reduce epidermal whitening produced in association with laser-assisted tattoo removal. This optical clearing agent has enabled multiple laser passes to be made in one treatment session. Previous studies using the PFD patch have showed enhanced clearance with picosecond and Q-switched lasers on blue/black tattoos in Fitzpatrick skin types I–III. We sought to explore the safety and efficacy of using the PFD patch with Q-Switched and picosecond lasers in Fitzpatrick skin types IV–VI.
Study Design/Materials and Method: A retrospective, single institution chart review was used to assess the safety of treating tattoos using the PFD patch with Q-Switched and picosecond lasers in Fitzpatrick skin types IV–VI. A total of 14 patients, ages 23–66 with Fitzpatrick skin types IV–VI were treated with the PFD patches and liquid PFD using the picosecond (532, 785 and 1064 nm) and the Q-switched Nd:YAG (1064 nm). The treated tattoos contained blue, black, red, green, purple and pink ink. Patient reported adverse events were evaluated.
Results: The PFD patch and liquid PFD were used with the picosecond (532, 785, 1064 nm) and the Q-switched Nd:YAG (1064 nm) lasers to treat tattoos safely in 14 patients with Fitzpatrick skin types IV–VI. Furthermore, the PFD patch was used safely when treating tattoo containing blue, black, red, green, purple and pink ink. Multiple passes were better tolerated in patients when using the PFD patch. No adverse effects were reported.
Conclusion: Our retrospective chart review supports the safety and efficacy of the PFD patch in protecting the epidermis from thermal injury during laser-assisted tattoo removal of various colours in patients with Fitzpatrick skin types IV–VI.

CLINICAL APPLICATIONS – CUTANEOUS: *VASCULAR*

EVALUATION OF THE EFFICACY AND SAFETY OF IPL USING A KTP FILTER FOR THE TREATMENT OF FACIAL TELANGIECTASIA

Jennifer D. Peterson, Daniel P. Friedmann
Suzanne Bruce & Associates, Houston, TX; Westlake Dermatology & Cosmetic Surgery, Austin, TX

Background: Both intense pulsed light (IPL) devices and KTP lasers achieve reduction of vascular lesions. An innovative IPL narrowband "KTP-like" filter (525 nm–585 nm) has been developed and combines the benefits of the well tolerated IPL with the precision of the KTP laser, for improved telangiectasia clearance. The aim of this study was to evaluate the impact of IPL KTP filter treatment on telangiectasia.
Study Design/Materials and Method: The study was a single center, prospective, open-label with a before-and-after study design. The study included 15 patients with Fitzpatrick skin types I–III, presenting with visible patches of facial telangiectasia. A total of three light treatments were conducted at monthly intervals with an IPL system (Lumenis Ltd.) equipped with a KTP filter (525 nm–585 nm). Three follow-up (FU) visits were performed at 1, 3, and 6 months following the last treatment session. Telangiectasia improvement was assessed by the investigator and the subject, using the 5-point clearance scale. Facial photographs were obtained on each visit. The level of the subject's discomfort was documented, using the visual analog scale (VAS), and subject's downtime was recorded. Subject's satisfaction was noted and a personal impression questionnaire was completed. Safety was monitored throughout the study duration.
Results: Following treatment, all facial telangiectasia showed improvement. Clinical improvement was evaluated by the investigator, on a per lesion basis, by facial area. Clinical improvement of chin telangiectasia ($n = 3$) at 1 month FU, was assessed as 3 (75–94% clearance), and ≥ 2 (over 50% clearance) for all lesions on the face ($n = 30$, excluding chin and forehead). Sixty-seven percent, 87% and 73% of the subjects were satisfied or very satisfied with the treatment at treatment 2, treatment 3 and 1 month FU. The study's acceptance criterion was met and therefore the study was deemed successful. No social downtime was evident after treatment 1, while mean social downtime of 2.3 and 3 days was documented following treatment 2 and treatment 3, respectively. The mean facial VAS scores documented throughout treatments were 3.5, 4.5 and 4.8 for treatment 1, 2 and 3 respectively.
Conclusion: The use of the novel IPL KTP filter produced a significant improvement of facial telangiectasia. The state-of-the-art combination of IPL technology with a narrowband filter can provide an effective alternative to KTP lasers and currently available wide spectrum IPL technology.

LASER TREATMENT OF PORT WINE STAINS IN INFANCY: A RETROSPECTIVE REVIEW OF 223 CASES

Hana Jeon, Daniel A. Belkin, Leonard J. Bernstein, Roy G. Geronemus
Laser & Skin Surgery Center of New York, NY

Background: The timing of the initiation of port wine stain laser treatment has been a source of controversy with variable opinions as to at what age treatments can safely and effectively begin. Recent concerns regarding repetitive use of general anesthesia under the age of three have placed greater importance on this controversy. Initiation of laser treatment during infancy has the potential to limit the use of general anesthesia and to facilitate improvement and complete clearing. We designed a retrospective study to evaluate the treatment success of port wine stains treated at the age of one year or younger.
Study Design/Materials and Method: A retrospective study of patients who received treatment for their port wine stains at our laser center between 2000 and 2017 at the age of one or younger was performed. Those who did not have follow-up photographs for evaluation were excluded. Chart reviews of 223 patients were performed to extract relevant data including demographic information, age at the time of procedure, and

treatment dates. Before and after photographs were reviewed by four physicians independently, and graded using the visual analog scale (VAS).

Results: Of the 223 patients (37% male and 63% female), majority (90%) had light skin types (I–III) and had facial lesions (80%), with the rest located on the trunk or extremities. The lesion size ranged from 0.36 to 800cm^2 (average 63.91). The treatment settings using the pulsed dye laser were 10–12 mm, 6.5–9 J, and 0.45–1.5 msec. The average age at the time of first treatment was 3 months (range 5–357 days) and the average number of treatment was 12 (range 1–48). Per the averaged physician VAS grading, 28.25% showed a 100% clearance, 44.39% showed 76%–99% improvement, 13.45% showed 51%–75% improvement, 7.62% showed 26%–50% improvement, and 6.28% showed 0–25% improvement. The patients did not experience scarring or pigmentary change.

Conclusion: Treatment of port wine stains in infancy is both safe and effective. Early intervention allows for treatment without general anesthesia, with faster and more complete clearance than what has been reported for treatments begun at older ages.

NOVEL PULSED DYE LASER FOR FACIAL REJUVENATION

E. Victor Ross, Srimanasi Javvaji, Jonathan Zumwalt, Hubert Chodkiewicz

Scripps Clinic, San Diego, CA

Background: A new pulsed dye laser has been developed for treatment of vascular and pigmented lesions of the face. The laser, compared to existing technologies, enjoys a larger spot size range, longer dye life, options for cryogen spray or contact cooling, and an option for radiofrequency energy deployment just before the laser pulse. This IRB approved study examined the role for this laser in facial rejuvenation.

Study Design/Materials and Method: The laser is a 595 nm pulsed dye laser. The spot size range for the study was 5-12 mm. Fluences ranged from 8–18 J/cm^2, with smaller fluences applied for general rejuvenation with the 12 mm spot (mean 9 J/cm^2). The smaller spot was used for (a) focal low contrast pigmented lesions and (b) vessels that persisted after overall facial treatment with the larger spot. Sapphire contact cooling was applied at 10°C. Cryogen spray cooling was not applied in full treatment sessions as it tended to overcool the epidermal pigmented lesions in test areas. A smaller area of the skin was reserved (typically pre auricular area) for addition of RF energy just before the pulse (40–70 J/cm^3) over 100 ms with a 20 ms delay between the end of the RF pulse and beginning of the laser pulse. The RF energy was applied via metal rails separated by 12 mm at the edge of the sapphire crystal. Generally, in all cases, the minimum fluence that achieved vessel closure and/or slight immediate pigment darkening, was applied based on test spots performed just before treatment to the entire face. Two treatments were performed one month apart with follow visits up to 3 months after the final treatment. Lidocaine cream (5%) was applied 30–60 minutes before all treatments.

Results: A total of 13 Caucasians (n = 12) and Hispanic (n = 1) subjects (9 females; 4 males) aged 61 ± 7 years old (range 51–74) with Fitzpatrick Skin type I–III (ST I n = 1; ST II n = 9; ST III n = 3) were enrolled in the study and were treated (mainly without RF) with contact cooling on 16 facial lesions. The lesions included diffuse redness (n = 3), photodamaged (n = 10) and telangiectasia (n = 3) coloured red and/or brown. All subjects underwent 2 treatment sessions except one who underwent 3 sessions. Mean discomfort during treatment was 4.7 ± 2.3 on a 0–10 scale (0 = no discomfort at all; 10 = intolerable pain). Following treatments immediate responses were mild to moderate erythema (100%), mild oedema (89%), mild purpura (33%) and mild hyper-pigmentation (8%). Two adverse events were observed (mild cold sores and moderate crusting). Two dermatologists blinded reviewers assessed before and 3 months after treatments photographs in random manner (for 13 lesions that arrived to 3m FU). Both assessed correctly all observations and ranked the clearance response using 5-point scale (0 = no change; 1 = 1–25% clearance; 2 = 26–50%; 3 = 51–75%; 4 = 76–100% clearance). All lesions had at least 50% clearance, while 77% had 50–75% clearance and 23% had 76–100% clearance. The application of RF energy did not affect the outcomes. Subject satisfaction rate was 91%, and at the same rate patients reported they would recommend this procedure.

Conclusion: A novel PDL is effective in one pass treatments for facial rejuvenation.

OPTICAL COHERENCE TOMOGRAPHY EVALUATION OF TOPICAL OXYMETAZOLINE HYDROCHLORIDE TREATMENT EFFICACY FOR ROSACEA

Hollie Ryan, Paul M. Graham, Daniel Woods, David H. McDaniel

McDaniel Institute of Anti Aging Research, Virginia Beach, VA; Michelson Diagnostics LTD, Maidstone, Kent, United Kingdom

Background: Due to its role in vasoconstriction, oxymetazoline hydrochloride is commonly used for nasal congestion and red irritated eyes. More recently, it was FDA approved for treatment of rosacea. A pilot study was performed to validate the efficacy of oxymetazoline in reducing erythema using optical coherence tomography (OCT) and utilizing new advanced image analysis to measure effects on erythema, roughness as well as vessel diameter at various depths in the dermis.

Study Design/Materials and Method: Informed consent was obtained from two rosacea subjects. A one-time application of 1% oxymetazoline hydrochloride cream was applied to the entire face. Digital images and 120 slice, 6 × 6 mm OCT scans were acquired at baseline and multiple time points up to 7 hours post-treatment, and again at either 1 or 2 days post-treatment. OCT images were analyzed for vessel diameter at various depths, changes in erythema, and skin roughness using multiple software programs.

Results: A significant reduction in vessel diameter occurred and then returned to baseline levels (±6%) at 1–2 day follow-up. Reductions in erythema from baseline (one subject) of 37.8% (left face) and 74.7% (right face) were observed (vessel diameter was noticeably greater on left than right). Vessel diameter at different depths were studied. Additionally, treatment produced mean roughness and surface height values that steadily decreased to 50–100% below baseline over 48 hours.

Conclusion: A one-time application of oxymetazoline created reduction in vessel diameter after application, and returned to baseline after 1–2 days. Decreased vessel diameter began during 1 hour post treatment, increasing to peak reduction between 6 and 26 hours. Oxymetazoline use may have important implications in laser surgery for parameter choice (for vessel diameter and washout period). Unexpectedly, skin roughness reduced by approximately 40% for up to 2 days and further testing is needed to compare vehicle versus oxymetazoline.

PULSED-DYE LASER TREATMENT OF ROSACEA USING A NOVEL LASER WITH A 15 mm DIAMETER TREATMENT BEAM

Eric F. Bernstein, Kevin Schomacker, Amit S. Paranjape
Main Line Center for Laser Surgery, Ardmore, PA; Syneron-Candela, Wayland, MA

Background: The pulsed-dye laser has been used to treat facial redness and rosacea for decades. Recent advances in dye laser technology enable 50% higher output energies supporting 25% larger treatment beam-diameters up to 15 mm with clinically-relevant fluences. In this study, we investigate this novel pulsed-dye laser using a 15 mm beam-diameter for treatment of rosacea.

Study Design/Materials and Method: Twenty subjects with erythemato-telangiectatic rosacea were enrolled in the study. A total of 4 monthly treatments were administered, first treating liner vessel with a 3 mm × 10 mm elliptical beam, then diffuse redness with a 15 mm circular beam. Blinded assessment of digital, cross-polarized photographs taken 8 weeks following the last treatment was performed using a 11-point clearance scale.

Results: Nineteen subjects completed all study visits. Blinded reviewers correctly identified baseline photos 55 out of the total of 57 cases (96.5%). The blinded reviewers scored 17 out of the 19 subjects with an improvement greater than 40% and 11 out of the 19 subjects greater than 50%. The average improvement was 53.9%. Investigator and subjects were very satisfied with the treatment results in 18 out of the 19 cases (94.7%) and somewhat satisfied in the one remaining case. Side effects were limited to mild oedema, mild to moderate erythema and mild to moderate bruising.

Conclusion: This study demonstrates that a new pulsed-dye laser having a novel 15 mm treatment beam improves the appearance of rosacea with a favorable safety profile.

USE OF A NOVEL 589 nm SOLID-STATE LASER FOR TREATMENT OF FACIAL ERYTHEMA

Diana Cohen, Bradley S. Bloom, David J. Goldberg
Skin Laser & Surgery Specialists of NY and NJ, Hackensack, NJ; Skin Laser & Surgery Specialists of NY and NJ, New York, NY

Background: For several decades, the gold standard for the laser treatment of vascular lesions has been the 595 nm pulsed dye laser (PDL). This laser, although highly effective, has cumbersome and costly maintenance requirements to replenish the dye medium. A brand new, novel solid-state laser that emits light at 589 nm has the potential to effectively treat vascular conditions without the maintenance issues that come with the PDL. This study examines the safety and efficacy of a 589 nm solid-state laser used for treatment of facial erythema.

Study Design/Materials and Method: This study is a single-center prospective cohort study of 30 subjects, 18 years of age or older, Fitzpatrick Skin Types I to IV, with varying degrees of facial erythema. Each subject received 4 full face monthly treatments with a solid-state 589 nm laser. Delivered fluences were 10–15 J/cm^2 with a 46 ms pulse duration using a scanning handpiece. Participants then returned for follow-up one month after the 4th treatment. Efficacy was evaluated by investigator and participant assessments of facial erythema utilizing a scale from 0 to 4 (0 = no erythema; 4 = severe erythema). Digital photographs were obtained at each visit. Safety was assessed by compiling investigator-reported side effects throughout the study.

Results: All subjects achieved at least 1 level of improvement. No complications were noted.

Conclusion: A new 589 nm solid state, non-rhodamine dye, solid state laser represents a novel approach for the treatment of vascular lesions.

CLINICAL APPLICATIONS – GYNECOLOGIC/ WOMEN'S HEALTH: *CO_2 LASER FOR VAGINAL APPLICATIONS*

EARLY EFFECT OF FRACTIONAL CO_2 LASER TREATMENT IN POST-MENOPAUSAL WOMEN WITH VAGINAL ATROPHY

Scott E. Eder
Center for Women's Health & Wellness, LLC, Plainsboro, NJ

Background: Fractional CO_2 lasers have been shown to provide improvement of vulvovaginal atrophy (VVA.) The aim of the current study was to assess the early effect of fractional CO_2 laser system in treating postmenopausal women with clinical symptoms of VVA.

Study Design/Materials and Method: Twenty-eight healthy post-menopausal women (mean age 60.1+/−5.55 years) with VVA-related symptoms were treated with fractional CO_2 laser 3 times, in 4-week intervals. At each study visit the Vaginal Health Index Score (VHIS) and VVA symptom severity were recorded. Sexual function was assessed with the Female Sexual Function Index (FSFI).

Results: One month following the first laser treatment VHIS was significantly improved (13.89+/ − 4.25 vs. baseline 11.93+/ − 3.82; $p < 0.05$,) and improved further at three and six months following all three laser treatments (16.43+/−4.20 and 17.46+/ −4.07.) Almost all VVA symptoms were significantly improved at one month following the first treatment. A further significant improvement in VVA symptoms was noted at three and six months following the third laser treatment. Following treatments, the FSFI score increased significantly (22.36+/ −10.40 vs. baseline 13.78+/−7.70; $p < 0.05$,) and remained significantly higher than baseline at the three and six month follow-up visits.

Conclusion: CO_2 laser therapy for post-menopausal women can be considered as an effective therapeutic option providing relief of symptoms noted already after one laser treatment.

IMPROVEMENT IN VULVOVAGINAL ATROPHY SYMPTOMS FOLLOWING FRACTIONAL CO_2 LASER

Stephen Swanson
Women's Clinic of Lincoln, P.C., Lincoln, NE

Background: Background improvement of vulvovaginal atrophy (VVA) has been reported following fractional CO_2 lasers. In our practice we assessed the effect of fractional CO_2 laser system in postmenopausal women with clinical symptoms of VVA.

Study Design/Materials and Method: Methods post-menopausal women (mean age 61 years) with VVA-related symptoms were treated with fractional CO_2 laser 2 times, in 4-week intervals. At each visit the Vaginal Health Index Score (VHIS) was evaluated by investigator and subject assessment vaginal atrophy were measured in a severity scale of 1–4 (1-absence of symptoms, to 4-symptoms as bad as it could be). Evaluations were done at baseline and following first and second treatment.

Results: Results Following the second laser treatment, the mean VHIS score was significantly improved compared to

baseline (18.7 ± 2.3 vs. 13.3 ± 2.4., $p < 0.0001$). Significant improvement in the subject assessment of vaginal atrophy was already noted following the first laser treatment and continued at the second one (3.2 ± 2.8 vs. 9.4 ± 3.1 $p < 0.001$). Almost all VVA symptoms were improved. Following two laser treatment 30% of the patient according to their definition were symptoms free. Follow-up of the third treatment in process. The treatment was well tolerated and produced no pain. No adverse events were reported.
Conclusion: Conclusion CO_2 laser therapy can be considered as an effective therapeutic modality for post-menopausal women suffering from VVA symptoms.

PIXELATED CO_2 LASER TREATMENT FOR GENITOURINARY SYNDROME OF MENOPAUSE: A PROSPECTIVE STUDY

Jeffrey Schachar, Laura Martin, David Ossin, Eric Hurtado, Guillermo W. Davila
Cleveland Clinic Florida, Weston, FL

Background: Genitourinary Syndrome of Menopause (GSM) includes many symptoms that negatively impact the lives of countless women. Vaginal estrogen has been the mainstay of therapy for the vaginal atrophy symptoms associated with GSM. Pixelated CO_2 laser therapy has been used in other fields of medicine for remodeling atrophic skin. Previous studies have demonstrated that laser therapy in gynecologic patients was safe.
Study Design/Materials and Method: Patients with symptomatic vaginal atrophy completed pre-treatment evaluations. The evaluation included a pelvic exam, vaginal pH, physician completed vaginal atrophy scoring evaluations [Bachmann Vaginal Health Index (BVHI), Vaginal Health Grade (VHG)], as well as several patient questionnaires [Vaginal Atrophy Bother Questionnaire (VABQ), Symptoms of Atrophic Vaginitis Questionnaire (SAVQ), Vulvovaginal Atrophy Symptom Questionnaire (VASQ), Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire (PISQ-12)]. Subjects underwent three treatments over 2 months. The treatments were standardized for all patients. Use of other treatments during the study period were prohibited. Subjects completed the same panel of assessments two weeks after each treatment. A $p < 0.05$ was considered statistically significant.
Results: Fifteen patients were enrolled with an average age of 58.3 years. Baseline evaluations were compared to the evaluations after the third treatment. Statistically significant improvements were noted on all three of the patient subjective questionnaires of vaginal atrophy [VABQ $p = 0.0001$; SAVQ $p = 0.0001$; and VASQ $p = 0.0002$], the sexual function questionnaire [PISQ-12 $p = 0.0045$], and both of the physician completed evaluations [BVHI $p = 0.0005$; VHG $p = 0.0001$].
Conclusion: With short-term follow-up clinically significant improvement in symptoms of Genitourinary Syndrome of Menopause was noted in those who underwent pixelated CO_2 laser therapy.

THE TREATMENT OF VAGINAL LAXITY USING A NOVEL FRACTIONAL MATRIX RADIOFREQUENCY

Sherry Thomas
The American Association of Female Pelvic Medicine Specialists, Agoura Hills, CA

Background: Female pelvic organ prolapse (POP), occurs in 50% of parous women; is the loss of support to the uterus, bladder and bowel. The connective tissue surrounding the pelvis organs composed of collagen, elastin, smooth muscles, and microfibers are all critical for pelvic floor support. Loss of collagen and elastin has been identified as a predominant cause of vaginal laxity. A method of treating vaginal laxity involves using radiofrequency (RF) energy to simulate collagen. The primary objective of this study is to assess the safety and efficacy of a novel fractional matrix radiofrequency for treating mild to moderate POP.
Study Design/Materials and Method: The study is an open label, non-randomized, observational, prospective study of 30 female subjects exhibiting signs of vaginal laxity. The subjects are treated 3 times at 4 week intervals using transvaginal fractional matrix radiofrequency. Objective measurement of the are assessed using the Baden-Walker Grading along with measurement of patient subjective symptoms using the VLQ, and GSA assessments collected at baseline, at weeks 4, 8, and 12, and 3 months after the final treatment. This presentation is an interim analysis of 20 patients at 90 days post third treatment.
Results: The patients show a statistically significant improvement in grade of prolapse, VLQ and GSA 90 days after three treatments with fractional RF. The adverse events included acute cystitis and vaginitis. Additionally, a few patients report a discharge post procedure lasting up to 3 days.
Conclusion: This clinical trial demonstrates efficacy and safety treating vaginal laxity using a novel fractional matrix radiofrequency with limited adverse events.

CLINICAL APPLICATIONS – GYNECOLOGIC/ WOMEN'S HEALTH: *COMBINED Nd: AND Er:YAG LASER FOR VAGINAL APPLICATIONS*

TREATMENT OF VULVAR PIGMENTATION USING THE COMBINATION OF Er:YAG AND Nd:YAG LASERS

Jorge E. Gaviria P., Aureni Betancourt, Zdenko Vizintin
Korpo Laser, Caracas, Venezuela; Fotona, Ljubljana, Slovenia

Background: Hyperpigmentation of vulvar area is quite common indication and represents aesthetic problem that influences the quality of life of such patients. Objective of our study was to evaluate the efficacy and safety of combined Nd:YAG and Er:YAG laser treatment for vulvar depigmentation.
Study Design/Materials and Method: Patients with a complaint of vulvar hyperpigmentation were included in this case series clinical trial. The treatment consisted of two phases. Firstly 2–3 passes of Nd:YAG 1064 nm laser (15 J/cm^2, 1.6 ms, 9 mm) across the pigmented area were delivered, followed by two passes of Er:YAG 2940 nm (1.1 J/cm^2, 0.1 ms, 7 mm) light peel of the same area. All patients received 3 treatment sessions with 4–6 weeks interval. No anesthesia was used. The efficacy was assessed by two independent evaluators on the basis of photographs, as well as with patient satisfaction questionnaire (0–10). Adverse effects were monitored at every visit and at one week after each treatment session.
Results: 60 patients with vulvar hyperpigmentation were included in this study and treated with combined laser depigmentation therapy in single center in Caracas, Venezuela in period from May 2015 to September 2016. Independent observers graded the average improvement as 2.2 ± 0.6 on the 0–3 scale (0 = no change, 1 = mild, 2 = moderate, 3 = excellent). Results of patient satisfaction were: 44 patients had satisfaction score of 7–10 points, 10 had 4–6 and 6 had 0–3 points at the 10 points scale. There were no adverse effects reported aside of

mild discomfort during the treatment and transient erythema, lasting up to one week.
Conclusion: Combined Nd:YAG and Er:YAG laser treatment of vulvar hyperpigmentation seems to be efficacious and safe method for this indication.

CLINICAL APPLICATIONS – GYNECOLOGIC/ WOMEN'S HEALTH: *ERBIUM LASER FOR URINARY APPLICATIONS*

A 12 MONTHS FOLLOW-UP STUDY OF ERBIUM LASER TREATMENT OF STRESS URINARY INCONTINENCE IN WOMEN USING ROBOTIC LASER PROBE

Adrian Gaspar, Sandra E. Maestri, Zdenko Vizintin
Prima Piel Clinic, Mendoza, Argentina; Fotona, Ljubljana, Slovenia

Background: The purpose of this clinical study was to evaluate the use of new robotic probe for the non-invasive erbium laser treatment for stress urinary incontinence in women.
Study Design/Materials and Method: This is a prospective case series study performed in one medical center from December 2015 to March 2017. SUI patients were treated with 3 sessions of non-ablative Er:YAG laser fitted with new robotic probe, once a month. Therapy efficacy was measured with ICIQ-UI questionnaire and 1 hour pad test at 3 and 12 months after the first session. No anesthesia was used for this therapy. After every treatment session as well as at every follow-up patients were interviewed about adverse effects and their vaginal canals were visually inspected.
Results: 40 patients having SUI of average age of 54.9 years (range 38–75), average BMI of 28.3 (range 24–41) and parity of 2.5 (range 0–8) were treated with 3 sessions of Er:YAG laser using robotic probe. Average ICIQ-UI score before the treatment was 11.7 points and on subsequent measurements after 3 and 12 months was 3.4 and 4.0 respectively. Average weight of urine loss measured with 1 hour pad test before the treatment was 9.6gr, at 3 months 4.2gr and at 12 months 6.9gr. Patients tolerated the treatment very well and were not reporting any discomfort with treatment. No adverse effects were reported by patients or observed by physicians.
Conclusion: The results of this study have shown that Er: YAG laser treatment with new robotic probe is efficacious in reducing stress urinary incontinence in women. Both measurement tools, ICIQ-UI and 1 hour pad test showed significant incontinence reduction at 3 and 12 months after the beginning of the therapy. Patients didn't report any discomfort during the treatment and their satisfaction with results was very high.

MIXED AND STRESS URINARY INCONTINENCE TREATMENT WITH VAGINAL ERBIUM LASER – COMPARISON OF LASER TREATMENT WITH TVT AND TOT IN ASIAN WOMEN WITH MUI AND SUI, 12 MONTHS FOLLOW-UP

Nobuo Okui, Kikue K. Yoda, Zdenko Vizintin
Yokosuka Urogynecology and Urology Clinic, Yokosuka, Kanagawa, Japan; Haltek, Musashino-shi, Tokyo, Japan; Fotona, Ljubljana, Slovenia

Background: Mixed urinary incontinence has components of stress and urge incontinence. Due to its complexity the treatment represents a challenge. This paper reports about assessment of efficacy and safety of non-ablative erbium laser thermal therapy in women with MUI and SUI in comparison to sling surgery TVT and TOT.
Study Design/Materials and Method: This was a prospective, single center study, on Asian women with mild-to-severe MUI and SUI performed between 2013 and 2016, in Yokosuka, Japan. Patients were divided into three groups and treated with: non-ablative Er:YAG laser (A), TVT (B) and TOT sling surgery (C). Laser therapy was performed with three sessions of vaginal non-ablative Er:YAG laser. Topical local anesthesia was used for laser and lumbar anesthesia for TVT and TOT surgeries. Efficacy for SUI was evaluated with 1 hour pad test and ICIQ-SF questionnaire and for MUI with OABSS questionnaire.
Results: 150 patients diagnosed with mild to severe SUI or MUI were included in this study and divided into Laser (A), TVT (B) and TOT (C) groups of 50 patients. There were 25 SUI and 25 MUI patients in A, 27 SUI and 23 MUI in B and 31 SUI and 19 MUI in C group. At 3 and 12 months follow-up results of 1 hour pad test and ICIQ-SF for SUI patients from all three groups were comparable improved, while the MUI patients improved only in laser group. All 25 MUI patients in A group improved, while of 42 MUI in groups B and C 17 patients worsened and 25 patients remained the same. There were no adverse effects in laser group, while there were severe pain, bleeding and infections present in groups B and C.
Conclusion: Non-ablative Er:YAG laser procedure seems to be superior to sling surgery for MUI and comparable for SUI at 12 months follow-up but with much less adverse effects.

THE FIRST EVALUATION OF ERBIUM LASER FOR TREATMENT OF FEMALE OVERACTIVE BLADDER SYNDROME IN COMPARISON TO STANDARD MEDICATION THERAPIES WITH 12 MONTHS FOLLOW-UP

Nobuo Okui, Kikue K. Yoda, Zdenko Vizintin
Yokosuka Urogynecology and Urology Clinic, Yokosuka, Kanagawa, Japan; Haltek, Musashino-shi, Tokyo, Japan; Fotona, Ljubljana, Slovenia

Background: OAB is a chronic condition of the bladder that causes sudden urges to urinate. In standard treatment, selective beta 3-adrenoceptor agonist and antimuscarinic drugs are used in eliminating urgency, but there are usually systemic side effects present. In this study we present the first assessment of the efficacy and safety of the non-ablative Er:YAG laser therapy on OAB symptoms and the comparison with standard therapies.
Study Design/Materials and Method: Three groups of postmenopausal Asian women with OAB were scheduled for: A) laser therapy, B) beta 3-adrenoceptor agonist and C) antimuscarinic drug therapy. Laser therapy was executed with vaginal Er:YAG 2940 nm laser operating in non-ablative mode designed to increase temperature of the vaginal mucosa up to 60−65°C. Beta agonist was administered one 25 mg tablet and antimuscarinic one 2.5 mg tablet per day. Efficacy was evaluated with the self-administered OAB questionnaire (OABSS) in comparison to beta 3-adrenoceptor agonist and antimuscarinic drug. In OABSS, efficacy was evaluated with total score, Q1 frequency, Q2 nocturia, Q3 urgency, and Q4 urge incontinence. Participants were evaluated at baseline, 1 month and 1 year after the last treatment.
Results: Study was executed in one clinic in Yokosuka, Japan between 2015 and 2017. 60 patients completed this study, 20 in each group. Mean ages of patients were: 64.2 (A), 65.4 (B) and 64.5 (C). In laser group OABSS scores decreased significantly (from 8.0 to 4.9) and there were

no side effects registered. In both antimuscarinic and beta 3 groups OABSS scores also decreased significantly (7.85 to 4.55 and 8.00 to 5.80). However, adverse events dry mouth and constipation occurred in most of the B and C subjects.
Conclusion: Results of erbium laser therapy of OAB were comparable to standard ones but with no adverse effects which are common for medication therapies.

CLINICAL APPLICATIONS – GYNECOLOGIC/ WOMEN'S HEALTH: *HIFEM TECHNOLOGY FOR URINARY APPLICATIONS*

HIFEM TECHNOLOGY – A NEW PERSPECTIVE IN TREATMENT OF STRESS URINARY INCONTINENCE

Red M. Alinsod, Vasil Vasilev
South Coast Urogynecology, Laguna Beach, CA; 2 Urology Cabinet 'Dr. Vasilev', Sofia, Bulgaria

Background: Stress urinary incontinence (SUI) is a prevalent condition among women and negatively affects their quality of life (QoL). The aim of the study was to assess the effect of High-Intensity Focused Electromagnetic (HIFEM) technology in the treatment of SUI.
Study Design/Materials and Method: 30 women with SUI were delivered a treatment course with HIFEM technology. Patients attended 6 therapies scheduled 2× a week. QoL was assessed through King's Health Questionnaire (KHQ) and amount of used hygienic pads. Data was collected pre-, post-treatment, at 3- and 6-month follow-up visits. Scores of the KHQ were calculated and statistically evaluated through t-test ($p < 0.001$). Amount of used hygienic pads were calculated as average.
Results: Course of the treatment with the HIFEM technology significantly improved QoL of all women. This was demonstrated as 77% level of improvement in incontinence impact according to the KHQ scores during 6-month follow-up. All patients significantly decreased the use of hygienic pads to 1.33 pad per day and night.
Conclusion: Results suggest that HIFEM technology is an efficacious therapy for treatment of SUI.

HIFEM TECHNOLOGY – THE NON-INVASIVE TREATMENT OF URINARY INCONTINENCE

Julene Samuels, Nathan L. Guerette
Julene B. Samuels, MD, FACS, Louisville, KY; The Female Pelvic Medicine Institute, Richmond, VA

Background: Urinary incontinence (UI) has a prevalence of 30–40% in post-partum and menopausal women and represents a very sensitive topic for those affected. Women may be reluctant to discuss the degree of their UI with their healthcare providers as well as the degree to which it may negatively impact their quality of life (QoL). Since UI has been reported to contribute to a decreased quality of life (QoL) for affected patients, presents a socio-hygienic problem for them, and seems to be a problem which lacks an efficacious non-surgical solution, we sought to quantify these findings and to report on a novel non-surgical treatment that may provide an affordable and efficacious non-surgical solution to this common problem.
Study Design/Materials and Method: In this pilot study of recently treated patients in two clinics in the United States (one plastic surgery clinic and one urogynecology clinic), we investigate the effectiveness of the treatment, as well as its impact on QoL of incontinent patients using a device that consists of High-Intensity Focused Electromagnetic Technology (HIFEM).
Results: 20 women (mean age of 59.47 years) who presented with stress, urge and mixed UI, were included in a pilot study using HIFEM technology (BTL Industries Inc.). All patients completed a total of 6 treatments performed twice weekly for 3 consecutive weeks. Patients completed Kings Health Questionnaires (KHQ) pre- and post-treatment. Additionally, patients reported on the frequency of urinary leakage episodes and number of used hygienic pads. This patient reporting was repeated during the 3- and 6-month follow-up visits to the clinics. Scores of the KHQ were calculated and statistically evaluated through t-test analysis ($p < 0.05$). Frequency of urinary leakage episodes and number of used hygienic pads were calculated through the patient-reported frequency of occurrence. The vast majority of the patients decreased the use of hygienic pads to minimum or totally eliminated them.
Conclusion: Results suggest that HIFEM technology significantly improves the QoL of post-partum and menopausal female patients who present with all types of UI.

CLINICAL APPLICATIONS – GYNECOLOGIC/ WOMEN'S HEALTH: *PDT/ PHOTOBIOMODULATION*

HISTOPATHOLOGICAL EVALUATION PRE AND POST TREATMENT WITH TOPICAL CORTICOSTEROID AND PHOTODYNAMIC THERAPY FOR VULVAR LICHEN SCLEROSUS

Renata Belotto, Raquel Civolani Marques Fernandes, Ana Carolina de Magalhães, Luciana Correa, M. Cristina Chavantes
Universidade Nove de Julho, São Paulo, Brazil; Perola Byington Hospital, São Paulo, Brazil

Background: The Vulvar Lichen Sclerosus (VLS) is a chronic dermatosis with possible autoimmune etiology. VLS affects anogenital region with intense itching causing minor vulvar lips and clitoral fusion leading to important quality of life impairment. The ultrapotent topical corticosteroids are the standard treatment, reducing inflammatory process and increasing disease-free intervals. Photodynamic therapy (PDT) is an alternative treatment which activates important inflammatory mediators and stimulates local immune effect, justifying its application in the VLS. The study main purpose is to demonstrate pre and post VLS histopathological response when applying PDT and topical corticosteroid.
Study Design/Materials and Method: Controlled and randomized study for VLS diagnosed histologically in 24 women, allocated in two groups: Corticoid Group (CG) and PDT Group (PDTG). CG: 0.05% clobetasol propionate ointment in 12 women, 24 hours/4 weeks and every 48 hours /4 weeks, totally of 8 weeks, were applied. PDTG: 1% of aqueous metilene blue solution intralesional and right after CW Diode laser, $\lambda = 660$ nm, $P = 100$ mW, $I = 510$ mW/cm^2, $E = 4$ J, $RA = 20$ J/cm^2, $T = 40$ seconds, were applied weekly for 8 weeks for 12 women. For both groups, the biopsies were performed for histological study pre and post 90 days treatments. The histological slides are classified according to the Robboy's classification VLS 1 (initial or stabilized) and VLS 2 (old).
Results: 11/12 (91.7%) patients in the CG pre-treatment, had VLS 2 and 1/12 (8.3%) patients presented VLS 1. In the post-treatment 4/12 (33.3%) patients maintained VLS 2 and 8/12 (66.7%) patients presented VLS 1. In the GPDT pre treatment, 11/12 (91.7%) patients had VLS 2 and 1/12 (8.3%) patient with VLS 1, while in the post treatment, 5/12 (41.7%)

patients maintained VLS 2 and 7/12 (58.3%) patients presented VLS 1.
Conclusion: The histopathological changed in both groups. PDT seems to resemble corticosteroid, focusing the histological aspect, and could be a VLS therapeutic alternative.

OUTCOME STUDY AFTER COMPARING PHOTODYNAMIC THERAPY AND EXCISION OF TRANSFORMATION ZONE FOR HIGH GRADE INTRAEPITHELIAL NEOPLASIA: 18 MONTHS OF OBSERVATION PROTOCOL

Renata Belotto, M. Cristina Chavantes, Fernanda Mansano Carbinatto, Cynthia Aparecida Castro, Raquel Civolani Marques Fernandes, Natalia Mayumi Inada, Vanderlei Salvador Bagnato
Universidade Nove de Julho, São Paulo, Brazil

Background: Cervical cancer is the 3rd most frequent tumour in the female population and the 4th cause of death worldwide. High grade intraepithelial neoplasia (HCIN) is the precursor of neoplastic lesion and the standard treatment of HCIN is excision of the transformation zone (ETZ). Photodynamic therapy (PDT) promotes photoinduced cell death and stimulates local immune effect, justifying its application in HCIN. The present study was to evaluate Thin prep (TP) or liquid cytology and PCR for high-risk HPV screening, in HCIN (CIN 2) patients treated by PDT and EZT, followed for 18 mo.
Study Design/Materials and Method: 24 women with HCIN (CIN 2) histological diagnosis, TP and PCR for HPV identification were collected pre- and post-treatment; with a follow-up for 18 months. The patients were allocated into 2 groups: ETZ Group (ETZG) and PDT Group (PDTG) with 12 women in each. All patients presented high grade lesion in TP pre treatment. TP and PCR were collected pre- and post-treatment, every 6 months for 18 months. ETZG: ETZ by an electrosurgical loop excision procedure electrode was performed, in order to remove the affected uterine cervix. PDTG: Methyl aminolevulinate cream 20% (MAL) was applied 10 hours before the procedure and then a LED, in single photo treatment, irradiated with λ: 630 nm, touching the cervix for 21 minutes, Fluence: 150 J/cm^2, Power density:120 mW/cm^2, was applied.
Results: In the ETZG pre-treatment 8/12 (66.7%) patients had positive DNA-HPV. After 18 months the TP remained positive in 2/12 (16.7%) patients and DNA-HPV in 3/12 (25%) patients. In the PDTG pre treatment, HPV DNA was positive in 8/12 (66.7%) patients and in 18 month follow-up, 4/12 (33.3%) patients remained TP positive for high grade lesion and 5/12 (41.7%) were HPV DNA positive for malignance high risk.
Conclusion: Post 18 months follow-up, the PDT showed to be a HCIN alternative treatment, by changing TP and PCR outcomes, without side effects or cervix removal.

VULVAR LICHEN SCLEROSUS: HISTOPATHOLOGICAL EVALUATION AFTER POST PHOTOBIOMODULATION TREATMENT AND TOPICAL CORTICOSTEROID

Renata Belotto, Raquel Civolani Marques Fernandes, Ana Carolina de Magalhães, Luciana Correa, M. Cristina Chavantes
Universidade Nove de Julho, São Paulo, Brazil; Perola Byington Hospital, São Paulo, Brazil

Background: Vulvar Lichen Sclerosus (VLS) is a non-neoplastic, non-infectious dermatosis of unknown etiology causing intense pruritus and stenosis of genital and urinary tract, and limiting sexual and social life. The standard treatment is the ultra-potent topical corticosteroid (CG), attempting the outbreaks. Photobiomodulation therapy (PBMT) can reduce inflammation; induce tissue reparation beyond immunomodulatory action. The study's aim was to compare the VLS histological response, pre and post treatment with topical steroid and PBMT.
Study Design/Materials and Method: Controlled and randomized study with 30 VLS patients diagnosed by histology, divided into two groups: CG: 0.05% clobetasol propionate ointment were applied in 15 women, every 24 hours for 4 weeks. Another 4 weeks, every 48 hours, totally 8 weeks treatment. PBMT: CW Diodo Laser w/ $\lambda = 660$ nm, P = 100 mW, I = 510 mW/cm^2, E = 4 J, RE = 20 J/cm^2, T = 40 seconds, weekly for 8 weeks were applied in 16 patients. For both groups, the biopsies were performed for Histological study pre and post 90 days treatments. The histological slides are classified according to the Robboy's classification VLS 1 (initial or stabilized) and VLS 2 (old).
Results: CG 13/15 (86.7%) pats in the CG had in pre-treatment VLS 2 and 2/15 (13.3%) patients with VLS 1. In the post treatment 4/15 (26.7%) patients maintained VLS 2 and 11/15 (73.3%) patients presented VLS 1. The pre-treatment of PBMT, 13/15 (86.7%) patients were diagnosed with VLS 2 and 2/15 (13.3%) patients with VLS 1, while in the post treatment treatment 5/15 (33.3%) patients maintained VLS 2 and 10/15 (66.7%) patients presented VLS 1.
Conclusion: The both groups resemble histologically at the post treatment, showing the inflammatory process reduction and the epithelial aspect improvement. Thus, PBMT can be a VLS alternative treatment.

CLINICAL APPLICATIONS – GYNECOLOGIC/ WOMEN'S HEALTH: *RADIOFREQUENCY FOR VAGINAL APPLICATIONS*

SAFETY AND MECHANISM OF ACTION OF NON-INVASIVE RADIOFREQUENCY TREATMENT FOR VAGINAL LAXITY: HISTOLOGICAL STUDY IN THE SWINE VAGINAL MODEL

David E. Kent
Dermatologic Surgery Specialists, Macon, GA

Background: Natural factors such as aging, childbirth, or hormonal changes during menopause can result in stretching and weakening of vaginal connective tissue. This can negatively impact women's quality of life. Structural changes in vaginal elastic fiber density have been evaluated with qualitative histologic studies. The objective of this study is to evaluate quantitative changes of the elastic fibers in the vaginal wall in a porcine model *in vivo* after volumetric radiofrequency heating by a RF intravaginal applicator (BTL Industries, Boston, MA).
Study Design/Materials and Method: An animal model was used (domestic pig, multipara: 5.67 ± 0.94 deliveries, 3 years). Three pigs were treated once per week for the course of three weeks. The treatments were performed on the vaginal canal area, using standardized BTL protocol. There were 2 follow-up evaluations (1 week and 1 month). At the end of each treatment and each follow-up session, biopsy specimens (by punch biopsy) and ultrasound video measurements of the vaginal wall thickness were obtained. The histologic samples were evaluated for collagen and elastin fibers quantity and number of fibrocytes. Statistical analysis using Repeated Measures ANOVA, Tukey-Kramer method and Friedman test were performed and had a significance level α set at 5% ($p \leq 0.05$).

Results: Statistical analysis showed a measurable increase of elastin ($p < 0.001$) and collagen ($p < 0.01$) fiber density after every treatment. The measured increase of elastic fibers was highest at the 1-week follow-up. Elastin at one-week follow-up accounted on average for $51.46 \pm 16.86\%$ of the tissue examined (increase of 36.5 percentage points when compared to the first measurement), while collagen accounted on average for 44.83 ± 18.92 % (increase of 17 percentage points). The number of activated fibroblasts also showed an increase ($p < 0.001$). Following the last treatment, the number of observed activated fibroblasts was on average 1782.17 ± 480.97. This measured a 16% increase compared to the initial treatment and a 274% increase compared to pretreatment evaluation. The results of vaginal wall thickness measurement did not show statistical significance ($p \leq 0.05$), although an increase of vaginal wall thickness by 1.66 mm (32%) was observed one week after the 3rd treatment.
Conclusion: Results of this study suggest that volumetric heating of vaginal tissue by a RF intravaginal applicator produced significant improvement in connective tissue organization in a porcine study. Neocollagenesis and neoelastogenesis were observed, along with increased number of activated fibroblasts.

TEMPERATURE CONTROLLED RADIOFREQUENCY ELECTROCOAGULATION FOR THE TREATMENT OF VULVOVAGINAL TISSUE LAXITY

Susan G. Murrmann
Methodist University and Baptist Memorial, Memphis, TN

Background: Vulvovaginal laxity is a symptom of pelvic floor dysfunction which causes a loss of strength and flexibility within the vaginal wall. It is frequently the consequence of aging, childbirth, or hormonal changes and is driven by changes in connective tissue. Vulvovaginal laxity can cause several untoward conditions including Stress Urinary Incontinence, decrease Sexual Satisfaction and Orgasmic Dysfunction resulting in practical, social, and quality of life issues. The aim of this study is to evaluate the clinical efficacy of temperature controlled radiofrequency electrocoagulation for the treatment of vulvovaginal tissue laxity through its heating effect which stimulates collagen and elastin. Other aims included assessment of safety, subject satisfaction, and change in sexual satisfaction.
Study Design/Materials and Method: 65 subjects with self-reported symptoms consistent with vaginal laxity and up to Baden-Walker Grade 2 or Stage 2 pelvic organ prolapse (≤ 1 cm past hymen) received 3 radiofrequency treatments at 30 day intervals, to the labia majora, mucosal surface of the vagina, and the vaginal walls. During each treatment, tissue temperatures were increased to 42°C–45°C and maintained for a minimum of 5 minutes per treatment area. Follow-up visits were performed at 3, 6 and 12 months post treatment. Efficacy and safety assessments were performed at each treatment and each subsequent follow-up visit. The primary endpoint was success on the Vaginal Laxity Questionnaire (VLQ). Additional outcome metrics included the 6-point Sexual Satisfaction Scale (SSQ), Female Sexual Function Index (FSFI), and a Global Satisfaction Questionnaire (GSQ). Pain related to the procedure and/or device and procedure related adverse events were evaluated at each treatment interval.
Results: This study demonstrated clinically and statistically significant improvements in vaginal laxity-VQL (mean improvement of 3.0 ± 1.2 maintained through 12 months), sexual satisfaction- SSQ (mean improvement of 1.0 ± 1.2 maintained through 12 months) and sexual function- FSFI (greatest improvement from baseline at 90 days, 7.2 ± 5.0, with further improvement at 180 days, change from baseline 8.3 ± 4.8). Satisfaction with ease of treatment, based on the GSQ was also quite high with 95% of the respondents being very satisfied with the treatment at 1 year and no subjects reported as being unsatisfied. Furthermore, 95% of the subjects responding to the questionnaire were satisfied with perceived vaginal tightening. Adverse events were mild and transient, with the most commonly reported event being erythema (8.63%).
Conclusion: Radiofrequency is safe and effective for the treatment of vulvovaginal laxity, and demonstrates success in improving sexual satisfaction and sexual function, with results maintained at one year after initial treatment.

THE TREATMENT OF ATROPHIC VAGINITIS USING A NOVEL SUPERPULSED FRACTIONAL CO_2

Sherry Thomas
The American Association of Female Pelvic Medicine Specialists, Agoura Hills, CA

Background: The Genitourinary Syndrome of Menopause (GSM) is a progressive, devastating condition. The limited transitory clinical effect of treatment using lubricants, herbs, and hormonal remedies urgently demands a revolutionary change for women suffering with this condition. Fractional CO_2 lasers have shown significant promise as a treatment for GSM, providing a longer more beneficial effect. The fractional CO_2 laser induces permanent changes by stimulating fibroblasts which produces new collagen and elastin in addition to increasing the blood flow of the vaginal epithelia layer. This treatment improves the symptoms of GSM by restoring the normal premenopausal physiological conditions of lactobacillus rich glycogen producing epithelial tissue.The primary objective of this study is to assess the safety and efficacy of a novel superpulsed fractional CO_2 laser for treating GSM.
Study Design/Materials and Method: The study is an open label, non-randomized, observational, prospective study of 30 post-menopausal female subjects exhibiting signs of vulvovaginal atrophy. The subjects are treated 3 times over 12 weeks using a CO_2 laser. This presentation is an interim analysis of 23 patients at 90 days post treatment.
Results: The visual analog scale for dyspareunia and the 6 domains of the FSFI scoring show a statistically significant improvement 90 days after three treatments with a CO_2 laser. The adverse events included acute cystitis and vaginitis. Additionally, some patients report an external genital irritation and others report a discharge post procedure lasting up to 7 days.
Conclusion: This clinical trial demonstrates significant evidence using a novel superpulsed fractional laser for treating GSM with limited adverse events.

CLINICAL APPLICATIONS – MULTI-SPECIALTY: *OPTICAL IMAGING*

FIRST CLINICAL RESULTS: OPTICAL SMARTPHONE-BASED ORAL CANCER SCREENING

Vania Firmalino, Afarin Golabgir Anbarani, Delaney Islip, Bofan Song, Ross Uthoff, Thair Takesh, Ron Liang, Petra E. Wilder-Smith
Beckman Laser Institute and Medical Clinic, *University of California, Irvine, CA; College of Optical Sciences, University of Arizona, Tucson, AZ*

Background: Because more than 2/3 of all oral cancers are detected after metastasis, clinical outcomes are poor, with 5-year survival rates approximating 20%. However, when oral cancers are

detected early, 5-year survival exceeds 85%. Thus, early diagnosis is crucial to ensuring good treatment outcomes and prognosis. Although effective screening of high-risk populations improves patient outcomes, conventional visual screening is ineffective. The overall goal of this project is to develop and evaluate the screening performance of a novel, low cost smart-phone-based mini-probe for oral cancer (OC) screening and monitoring of oral potentially premalignant lesions (OPMLs). The device uniquely provides high resolution dual-modality white light images (pWLI) in combination with auto fluorescence imaging (AFI) capability. The aim of this study is to evaluate pWL and AF images of healthy oral mucosa, OPMLs, and OC to identify typical optical characteristics of each, develop and improve a diagnostic algorithm, determine diagnostic cutoffs and finally to evaluate diagnostic performance.
Study Design/Materials and Method: 92 subjects with visually healthy oral mucosa, or with areas of oral leukoplakia, erythroplakia or ulceration were recruited in compliance with UCI IRB 2002–2805. PWL and AF as well as standard photographic images were recorded of 8 standard locations in the oral cavity of each patient. Each of these locations was separately diagnosed per usual standard of care by a blinded pre-standardized clinician. By evaluating a total of 32 variables from the pWL and AF images, characteristic signatures and cutoffs were determined for healthy mucosa, OPMLs and OC at each of the standard locations.
Results: Inter-subject variation at each location was small, but inter-site differences were considerable. Optical data from OPML and OC sites differed from normal with regard to white-light reflectance intensities, vascular homogeneity and standard deviation. The AFI signal in OPMLs and OC shifted progressively to the red, together with a diminished green fluorescence signal. The cloud-based diagnostic algorithm based on these properties performed well, with an agreement with standard-of care diagnosis (kappa value) of 80.6%.
Conclusion: A low-cost compact oral probe combined with cloud-based diagnostic algorithm was able to differentiate between healthy, dysplastic and malignant oral mucosa. This project was supported by funding from 1R03EB014852, UH2 EB022623, P41EB015890 and the Beckman Foundation.

CLINICAL APPLICATIONS – MULTI-SPECIALTY: *PDT*

INDYGO: FOLLOW-UP AND PRELIMINARY RESULTS OF THE FIRST EVER PILOT CLINICAL TRIAL ON INTRAOPERATIVE 5-ALA PDT FOR THE TREATMENT OF NEWLY DIAGNOSED GLIOBLASTOMA

Maximilien Vermandel, Clément Dupont, Fabienne Lecomte, Pascal Deleporte, Serge R. Mordon, Nicolas Reyns
University of Lille and Image Assisted Laser Therapy for Oncology, Lille, France

Background: Glioblastoma (GBM) is a malignant brain tumour with a median overall survival of approximately 15 months with the current standard of care (SOC). Although GBM is a rare neoplastic disease with low prevalence (0.3/10,000 persons), it remains the most frequent primary malignant brain tumour in adults. Currently, no existing therapeutic agent is able to stop GBM progression, and complete resection is rarely feasible, since tumour cells usually infiltrate the surrounding brain: adjuvant therapies to improve local control are thus highly expected. Recently, 5-ALA interstitial photodynamic therapies have been reported with promising results. However, if one consider the absence of controlled clinical trial, efficacy of 5-ALA PDT is not still evidenced and thus not included in the standard protocol. We present here an ongoing clinical trial (INDYGO) to evaluate 5-ALA PDT delivered intraoperatively to treat newly diagnosed GBM.
Study Design/Materials and Method: Our group has previously introduced a specific light applicator to deliver PDT in the surgical cavity early after maximal resection to demonstrate 5-ALA-PDT efficacy on newly diagnosed GBM. With this concept, intraoperative PDT becomes a seamless treatment strategy easily embeddable into the standard surgical protocol. In this context 5-ALA PDT is delivered in combination with the SOC recommended by the current guidelines and enabling to simultaneously investigate the potential synergistic effects.
Results: In four months, five patients have already been treated with this new technology, five patients remain to be enrolled. Currently, therapy has been delivered without significant toxicity or adverse event and are fulfilling the primary endpoint of this feasibility study. Secondary endpoints still being under investigation are progression-free survival, overall survival and patients' quality of life.
Conclusion: Finally, after the feasibility and the absence of adverse effects, multicentric, parallel-group, randomized controlled trial (RCT) is planned to assess the efficacy of 5-ALA PDT for the treatment of newly diagnosed GBM.

CLINICAL APPLICATIONS – MULTI-SPECIALTY: *PHOTOBIOMODULATION*

ANALYZING ORAL WOUND HEALING ON MURINE MACROPHAGE CELL LINES USING PHOTOBIOMODULATION

Toneia Washington, Saeed Ur Rahman, Sasikumar Ponnusamy, Praveen Arany
University at Buffalo School of Dental Medicine, Buffalo, NY

Background: Wounds affect around 6.5 million patients with an estimated $25 billion yearly funds for treatment. An increase in the aging population and incidence of metabolic diseases such as diabetes and obesity are significant factors that affect wound healing outcomes. Photobiomodulation (PBM) therapy is the use of low dose biophotonics devices to reduce pain and inflammation and improve wound healing.
Study Design/Materials and Method: In this study, we used PBM therapy to determine its anti-inflammatory effects on proliferation and migration of murine macrophage (Raw 264.7) cells in vitro. It is treated with a bacterial cell wall component, lipopolysaccharides (LPS) to simulate infection. The proliferation assay was performed with a fluorescence assay, Alamar blue, that helps determine changes in cell numbers (proliferation). Cell migration was examined by scratching (wounding) a confluent cell layer and assessing their ability to move towards each other over 24 hours. In each of these studies, blue (450 nm) and red (650 nm) lights were used to examine the effect of two popular PBM clinical therapy wavelengths on proliferation and migration of these cells.
Results: Our results showed that PBM therapy was capable of increasing proliferation and migration of macrophage cells. Additionally, when cells were treated with PBM in the presence of LPS, cell migration was clearly increased compared to LPS treatments alone. These results suggest that PBM therapy is able to promote a more efficient inflammatory response in wounds to improve resolution and promote tissue healing. This would be clinically significant in burns or infected wounds.
Conclusion: In conclusion, PBM therapy offers a novel clinical therapy to promote wound healing by modulating the inflammatory response.

CAN PHOTOBIOMODULATION THERAPY IMPROVE BRONCHIAL HYPER-RESPONSIVESNESS IN ASTHMATIC PATIENTS: A CLINICAL STUDY

Clariana Rodrigues Soares, Nathali Cordeiro Pinto, M. Cristina Chavantes, Alana Dias, Auriléia A. Brito, Ana Paula Ligeiro Oliveira

University of Nove de Julho, São Paulo, Brazil

Background: Globally, bronchial asthma distresses more than 300 million people and poses a large socioeconomic burden, by 2025 over 100 million new asthma patients will be develop it worldwide. The morbidity and mortality rate remain at unacceptable levels, associated hospitalization rate and medical costs are still on the rise as well. Overreliance on medication and associated adverse drug effects have led to exploration of alternative management modalities. Several experimental studies against bronchial hyper-responsiviness for lung inflammatory diseases had been applied low-level laser therapy (LLLT). The present study was to evaluate photobiomodulation (PBM) response in patients with moderate and severe bronchial asthma. A prospective, descriptive, longitudinal, non-controlled study with diagnosis of bronchial asthma for at least 3 years, using bronchodilators medicines, in which patients are their own controls.

Study Design/Materials and Method: A CW Diode Laser applying a 660 nm wavelength, P = 35 mW, ED = 26,3J/cm^2, Time = 30 sec irradiation for each point inside the mouth as well as totaling 22 pts on the face, using λ = 780 nm, P = 35 mW, ED = 26,3J/cm^2, t: 30 sec irradiation for each point was delivered for each patient. The PBM treatment were twice a week during 5 weeks of total applications. All patients after 5 sessions underwent to sputum examination by ELISA to flow cytometry and cytokines analysis were reevaluated.

Results: Patients referred less bronchial symptoms post laser treatment free of bronchial crises during all period and a month later. The outcome indicated that LLLT was able to decrease, significantly, the macrophages, eosinophils, IL-8 and enlarged the production of IL1-β, IL-6, IL-10, IFN-γ levels as well as increased CD4 T lymphocytes in induced sputum. Besides, there was a rise in the peak expiratory flow by spirometry.

Conclusion: The photobiomodulation therapy improved patients' asthma clinical symptoms and signs, without side effects, non-invasive and cost-effectiveness treatment.

EFFECTS OF PHOTOBIOMODULATION THERAPY IN SURGICAL WOUND HEALING OF ABDOMINAL WALL HERNIORRHAPHY: A PILOT STUDY

Fabrício S. da Silva, Alecsander Ojea, Marcos V. B. Cezillo, Marcos R. Monteiro, Luz Marina A. Oliveira, Ana Carolina de Magalhães, M. Cristina Chavantes

Universidade Nove de Julho, São Paulo, Brazil; Antônio Giglio Hospital, São Paulo, Brazil

Background: Abdominal wall hernias are still a challenge for patients and physicians, due to high rate recurrences, which are associated with increasing morbidity and high costs. Abdominal wall hernias are due to elevation of intra-abdominal pressure resulting in bowel protrusion. Polypropylene prostheses have been applied although it may cause allergic/hypersensitivity reactions, inflammatory response and tissue-repair problems. The study's aim was to evaluate in patients photobiomodulation therapy (PBMT) on healing and pain post herniorrhaphy.

Study Design/Materials and Method: A randomized, controlled pilot study, in which 5 patients were divided into Low-Level Laser Therapy (LLLTG-3) and Placebo (PG-2) Groups were submitted to Laser-on and off, respectively. A CW Diode Laser w/660 nm wavelength, Power: 100 mW, Energy: 3 J/pt, Exp Rda: 50 J/cm2, PD: 982 W/cm2, Time: 10 s and Fluence: 33 J/cm2 on aponeurosis, previous the non-absorbable mesh were fixed and cutaneous area sutured, intraoperatively, totalizing 14 pts in the abdominal area. In the immediate postoperative (PO) period catheter were left into the wound bed for collect secretion and evaluate the inflammatory process. At 1st and 3th PO onto 7pts the LLLT were applied. A clinical evaluation of the surgical wound were performed pre each laser application. In 7th and 14th PO were re-evaluated for intercurrences signs (seroma, dehiscence, local inflammation and infection).

Results: Patients in the LLLT G had a better clinical response, w/ decreased phlogistic signs: as pain, edema, hyperemia, seroma and pruritus reduction surrounding the surgical wound compared to PG. LLLTG dropped 3 folds in lactic dehydrogenase (DHL) and creatine kinase (CK) levels from catheter secretion indicating better control of inflammatory and healing process.

Conclusion: PBMT provided a diminish in inflammatory clinical signs and biochemical analysis compared to Placebo. Laser application aided reducing and preventing postoperative complication (seroma, surgical peri-wound pruritus and other phlogistic/allergic signs). In addition, there was a decline in pain (analgesic effect) and cicatrization improvement by this preventive procedure.

PHOTOBIOMODULATION THERAPY OVER FLOW-MEDIATED DILATION IN POLICE OFFICERS: A PILOT STUDY

Cleber R. Alves, Jose R. de Moura, Jose Ribeiro Lemos Junior, M. Cristina Chavantes, Camila Paixão Jordão, Ivani Trombetta

Universidade Nove de Julho, São Paulo, Brazil

Background: Stress, a daily condition of the police officers (PO) in Brazil, has been implicated in cardiovascular diseases. Endothelial dysfunction is an early marker of cardiovascular risk. Previous studies have shown that low-level laser therapy (LLLT) promotes increased vascular reactivity. To determine whether PO have endothelial dysfunction and if the photobiomodulation therapy by low-level laser therapy LLLT applying would improve the vascular reactivity.

Study Design/Materials and Method: We studied 12 POs, with age between 35 and 50 years (75 ± 10.3 kg) that were randomized into 2 groups: PO that remained without any therapy for 3 months as a control period (C, n = 6); and PO submitted to low level laser therapy for 3 months (LLLT, n = 6). LLLT was applied twice weekly in the sublingually, with a maximum of three days between one application and another. Cardiovascular hemodynamics were evaluated at rest. The studied variables were heart rate (HR), mean blood pressure (MBP), brachial shear rate (SR) and brachial artery flow-mediated dilation (FMD).

Results: There were no differences between Pre and Post period in both groups, C and LLLT, in the HR (72 ± 4 vs. 74 ± 1, and 73 ± 2 vs. 70 ± 4 bpm, respectively, p = 0.35), MBP (93 ± 2 vs. 94 ± 1, and 91 ± 2 vs. 93 ± 1 mmHg, respectively, p = 0.15) and SR (43078 ± 4876 vs. 37770 ± 6194, and 47589 ± 5657 vs. 30963,4 ±1133,6 AUC, respectively, p = 0.15). Interestingly, C did not change and LLLT had brachial artery FMD enhanced from Pre to Post period (4.7 ± 4.5 vs. 6.2 ± 4.6 and 3.4 ± 2.5 vs. 22.9 ± 3.3 %, respectively, interaction effect = p = 0.02),

demonstrating an improvement of 16% after LLLT. Ours principal finding was that LLLT lead to significant increase on brachial artery FMD, an important marker of endothelial function.
Conclusion: Our data can indicate that chronic photobiomodulation therapy could improve the flow-mediated dilation, increases and restores conduit artery function in stressed police officers in Brazil.

TREATMENT OF CUTANEOUS METASTASES OF BREAST CANCER WITH SNET2 BASED PHOTODYNAMIC THERAPY

Thomas S. Mang, Ron R. Allison, Frank G. Pilkiewicz, Felix Garzon, Steve Rychnovsky
University at Buffalo, Buffalo, NY; 21st Century Oncology, Greenville, NC; Adgero Biopharmaceuticals, Princeton, NJ

Background: Metastatic breast cancer often gains resistance to cytotoxic treatments and is generally considered an incurable condition. Among visceral cancers, it's also the one most likely to develop cutaneous metastases. Progression of such CMBC tumours can lead to fungating masses that significantly decrease patient quality of life. PDT with the photosensitizer SnET2, product candidate REM-001, has demonstrated promising local control of CMBC with minimal morbidity in patients who have failed multimodality treatment schemes. therapy.
Study Design/Materials and Method: The study was designed to determine rate of clinical success of SnET2 PDT in the treatment of CMBC in patients who have failed prior radiation therapy and progressed on systemic therapy. 68 patients meeting inclusion/exclusion criteria were entered into two Phase 2/3 clinical trials labeled CA008 and CA009. Patients received 1.2 mg/kg infusions of REM-001 followed by light activation (200 J/cm^2 at 664 nm) of the CMBC sites at 24 hours post-infusion. Treated lesions were assessed monthly and scored by overall Clinical Success, a per-patient efficacy measure consisting of the fraction of evaluable lesions that responded minus the fraction of non-responders. Evaluable lesions were classified as those for which post-treatment PDT effects had fully resolved.
Results: Twenty patients continued in each study had evaluable lesions. The clinical success rate was 60% (95% CI: 39–81) in study CA008 and 50% (95% CI: 28 – 72) in study CA009.
Conclusion: Prior analyses have shown that SnET2 therapy is effective at inducing necrosis in individual lesions. The Clinical Success figure-of-merit used in this analysis was an assessment measure agreed to between the prior sponsor and FDA as a means to assess overall patient benefit. These results indicate SnET2 based PDT is a promising treatment for overall control of CMBC in patients who have failed prior treatment. This treatment may be useful in preventing such tumours from advancing to become highly symptomatic.

CLINICAL APPLICATIONS – MULTI-SPECIALTY: *PHOTOBIOMODULATION (DENTAL)*

DOUBLE-BLINDED STUDY TO INVESTIGATE EFFECTIVENESS OF 980 nm DIODE LASER FOR CHRONIC PERIODONTAL DISEASE – PRELIMINARY RESULTS

Inder S. Raj Makin, Robert Levine, Marc Shlossman
A.T. Still University, Mesa, AZ

Background: This double-blinded pilot study evaluates clinical effectiveness of a 980 nm diode laser in combination with scaling-and-root-planing (SRP), versus SRP alone for chronic periodontal disease patients. Most laser application reports for periodontal disease management rely on a split-mouth study, whereby the patient's laser-treated oral-cavity half is compared to the control half. In this 15-patient, 2-armed study, each patient was randomly selected to receive either SRP with diode-laser treatment, or SRP with sham laser treatment. Comparison was made between two distinct patient treated study arms.
Study Design/Materials and Method: Within an academic dental clinic, patients with moderate to severe chronic periodontitis were recruited. Important inclusion criteria were, pocket depth of greater than 5 mm in at least 4 teeth within 2 quadrants. Key exclusions were, uncontrolled diabetes and excessive cigarette smoking (greater than 10 daily). Included patients were randomly placed in the group receiving SRP in combination with a diode laser treatment or SRP with sham laser treatment. The laser system had unique capabilities to operate at constant temperature. A multi-step laser delivery procedure was implemented to optimize energy-tissue interaction. Following periodontal treatment, patient follow-up was at 2, 6, and 14 weeks. Periodontal charting, standardized intra-oral photography, and subgingival bacterial samples were acquired from randomly selected teeth. In each case the periodontal specialist and patient were masked to type of treatment delivered.
Results: Following SRP with sham laser intervention, average pocket depth in targeted teeth reduced by 6.4% compared to active laser group (12.5%) over a 14-week period. The declining trend for pocket depths at, 2, 6 and 14 weeks is faster for active laser group compared to sham-treated group.
Conclusion: Based on preliminary analysis, SRP in combination with customized diode laser treatment is potentially more effective in improving periodontal status compared to SRP alone, for chronic periodontitis.

CLINICAL APPLICATIONS – MULTI-SPECIALTY: *RADIO FREQUENCY*

A TWO STEP APPROACH USING A RADIOFREQUENCY DEVICE TO TREAT DRY EYE SYMPTOMS ASSOCIATED WITH MEIBOMIAN GLAND DYSFUNCTION AND CONJUNCTIVOCHALASIS

Edward Jaccoma
New England Dry Eye, Kittery, ME

Background: Dry eye has multiple etiologies, but two of the most prevalent are Meibomian Gland Dysfunction (MGD) and Conjunctivochalasis (CCH). This study examines how a strategy that treats two prevalent conditions concurrently can provide patients with comprehensive and customizable relief from dry eye.
Study Design/Materials and Method: A retrospective chart review of 25 patients was conducted. Patients were included if they presented with symptoms of dry eye and were treated with a single 4 MHz Monopolar Radio frequency (RF) System with a combination treatment approach to improve both MGD and CCH. MGD was treated using a 10 mm aesthetic handpiece to massage and heat the glands to a target temperature of 42 degrees. CCH was treated in CUT mode using a surgical handpiece and micro-insulated ball tip electrode to perform the plication treatment. Follow-up visit information was compared to Baseline to evaluate efficacy and safety of treatment.

Results: Standard patient evaluation of eye dryness (SPEED) total decreased by 34.6% and subject responses indicated that 83% of the subjects noted improvement. The total number of meibomian glands yielding liquid secretion (MGYLS) increased from 137 to 206 (34%). The number of MGYLS producing moderate or copious amounts of tear oil increased from 25% at baseline to 49% at follow-up. Noninvasive Tear break-up time (NTBUT) of the first tear increased by an average of 2.3 ± 6.3 seconds (45%). Schirmer test results increased $2.3\text{mm} \pm 6.9$ (20%). SPEED, MGYLS, NTBUT and Schirmer test results were statistically significant ($p < .05$) as compared to baseline. Evaluation of photographs showed marked improvement in CCH. Side effects were minimal. Common side effects seen were tenderness and irritation, which subsided within 2–5 days and redness, which subsided within 2–4 weeks.
Conclusion: The use of a two-step approach using a radiofrequency device treat dry eye symptoms provides significant improvement and relief for a broad spectrum of patients with dry eye.

EARLY CAREER: *BASIC SCIENCE*

ANTIMICROBIAL PHOTOTHERAPY USING BLUE LASER LIGHT: A PROMISING STRATEGY TO CLEAR WOUND INFECTIONS

Katia Rupel, Luisa Zupin, Giulia Ottaviani, Matteo Biasotto, Serena Zacchigna
University of Trieste, Trieste, Italy

Background: The development of novel antimicrobial strategies has returned of crucial importance due to increasing resistance towards conventional antibiotics. The aim of the present study was to evaluate the antimicrobial effect of blue laser light on Pseudomonas aeruginosa (PAO) *in vitro* and *in vivo*.
Study Design/Materials and Method: A multiple wavelength diode laser device was employed. To evaluate the antimicrobial efficacy of blue laser (445 nm) and select the most effective protocols, multiple screening sessions have been performed employing several irradiation protocols in terms of power density and fluence on PAO grown in planktonic state and on agar plates. The protocols showing the best antimicrobial activities have been then applied on eukaryotic cell lines (human keratinocytes and fibroblasts) to evaluate possible toxicity. All the experiments have been conducted testing also a red (660 nm) and infrared (970 nm) laser wavelength. The effect of laser irradiation has been evaluated also on mature PAO biofilms grown in flow cells visualized in 3-dimensional reconstructions using laser scanning confocal microscopy. The best protocol in terms of antimicrobial efficacy and low toxicity was then employed *in vivo* on a mouse model of skin wound infection. The possible involvement of oxidative stress in the mechanism of action has also been investigated. Furthermore, a blue LED light source was tested for possible differences.
Results: Among the different laser wavelengths tested with multiple protocols, only the blue ones showed a significant antimicrobial effect ($p < 0.0001$). The irradiation protocol with power density $0.3\,\text{W/cm}^2$ and fluence $60\,\text{J/cm}^2$ was selected, confirming its efficacy and low toxicity *in vivo* ($p < 0.01$). Oxidative stress seems to be involved in the blue light-mediated antimicrobial mechanism. Blue LED light showed a similar but slightly lower antimicrobial effect.
Conclusion: The use of antimicrobial phototherapy employing blue laser light has the potential to become a concrete treatment option for super infected wounds.

IN VIVO OPTICAL IMAGING OF TOPICALLY APPLIED GOLD MICROPARTICLES IN ACNE AND HEALTHY SKIN

Christine Fuchs, Vinzent K. Ortner, Peter A. Philipsen, Mette Mogensen, Merete Haedersdal
Bispebjerg University Hospital, Copenhagen, Denmark

Background: Reflectance confocal microscopy (RCM) and optical coherence tomography (OCT) are bedside imaging technologies with micrometer resolution and visualization depths of 200–300 μm and 1–2 mm, respectively. Recently, a treatment using gold microparticles as a chromophore to generate selective photothermolysis of pilosebaceous units has been developed. To date, histopathologic analysis of biopsy tissue has been the only available tool to evaluate gold microparticle (GMP) delivery in skin. The aims of this study are to use RCM and OCT to describe biodistribution of topically applied gold microparticles (GMP) in acne-prone and healthy skin and to evaluate the potential of GMP as contrast enhancing agent.
Study Design/Materials and Method: Acne patients ($n = 14$) and healthy participants ($n = 7$) were enrolled. Gold coated silica microparticles (150 nm) were massaged into facial skin. Cross-sectional OCT images and en face RCM images of epidermis and dermis were obtained. Morphological image features were described and in RCM the fraction of pilosebaceous units containing GMPs in epidermis, at the dermo-epidermal junction and in dermis was estimated.
Results: GMP enhanced contrast of hair follicles in all images. In RCM images, the GMPs appeared as hyperreflective dots and aggregates inside hair follicles and eccrine ducts. With OCT, GMPs lined hair follicles and showed as enhanced hyperreflective structures. GMPs were only detected within the pilosebaceous unit and not in surrounding skin. In OCT images, GMPs were detected to a maximum depth of 1080μm and a mean depth of 545 ± 220 μm. Fraction of hair follicles containing GMPs at various skin depths were calculated.
Conclusion: This study demonstrates the feasibility of RCM and OCT to visualize topically applied GMPs in the epidermis and dermis. GMPs are confined to hair follicles and eccrine ducts where GMPs exhibit hyperreflectivity.

POTASSIUM IODIDE POTENTIATES ANTIMICROBIAL PHOTODYNAMIC INACTIVATION MEDIATED BY TPPS4

Liyi Huang
Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA

Background: We recently reported that addition of the non-toxic salt, potassium iodide can potentiate antimicrobial photodynamic inactivation of a broad-spectrum of microorganisms, producing many extra logs of killing. If the photosensitizer (PS) can bind to the microbial cells, then delivering light in the presence of KI produces short-lived reactive iodine species, while if the cells are added after light the killing is caused by molecular iodine produced as a result of singlet oxygen-mediated oxidation of iodide.
Study Design/Materials and Method: In an attempt to show the importance of PS-bacterial binding, we compared two charged porphyrins, TPPS4 (thought to be anionic and not able to bind to Gram-negative bacteria) and TMPyP4 (considered cationic and well able to bind to bacteria).
Results: As expected TPPS4 + light did not kill Gram-negative Escherichia coli, but surprisingly when 100 mM KI was added, it was highly effective (eradication at $200\,\text{nM} + 10\,\text{J/cm}^2$ of

415 nm light). TPPS4 was more effective than TMPyP4 in eradicating the Gram-positive bacteria, methicillin-resistant Staphylococcus aureus and the fungal yeast Candida albicans (regardless of KI). TPPS4 was also highly active against E. coli after a centrifugation step when KI was added, suggesting that the supposedly anionic porphyrin bound to bacteria and Candida. This was confirmed by uptake experiments.
Conclusion: We conclude that TPPS4 behaves as if it has some cationic character in the presence of bacteria, which may be related to its delivery from suppliers in the form of a dihydrochloride salt.

THE ABLATIVE FRACTIONAL COAGULATION ZONE IMPACTS UPTAKE OF SODIUM FLUORESCEIN AND CARBOXY FLUORESCEIN IN SKIN – AN *IN VITRO* STUDY

Christina Banzhaf, Vinzent K. Ortner, Peter A. Philipsen, Merete Haedersdal
University of Copenhagen, Bispebjerg Hospital, Copenhagen, Denmark

Background: Ablative fractional laser (AFXL) increases uptake of topical agents in skin. The coagulation zone (CZ) surrounding vertical ablated channels may impact drug uptake. Sodium fluorescein (NaF) and carboxy fluorescein (CaF) (370 MW, log PNaF −1.52, log PCaF 2.0), two fluorescent molecules may simulate small hydrophilic and lipophilic drugs. Our aim was to investigate uptake of NaF and CaF in pretreated skin with various CZ thicknesses by fluorescence confocal microscopy (FCM) and fluorescence microscopy (FM).
Study Design/Materials and Method: After four hours incubation, FCM (n-total = 216 scans) quantified fluorescence intensities (FI) of NaF and CaF with 488 nm excitation in CZ and surrounding skin to a depth of 90 μm. FM (ntotal = 1272 sections) assessed NaF FI at selected skin depths from 0 to 1500 μm. Micro channels with CZ thicknesses ranging from 0 to 80 μm were generated from micro-needles and AFXL (10,600 nm). Channels were 700 μm deep and number of channels kept consistent per skin area.
Results: FI of NaF and CaF differed and depended on CZ thickness and skin depth. By FCM, NaF FI reached higher levels in pretreated skin with a CZ compared to pretreated skin with no CZ ($p < 0.02$), whereas CaF FI reached higher FI in pretreated skin with no or a thin CZ ($p < 0.02$). FM supported NaF data showing highest FI in pretreated skin with a thin CZ ($p < 0.042$). FCM technique, showed decreasing FI with skin depth (Stratum corneumCZ-20 + NaF: 70 AU (49–112), EpidermisCZ-20 + NaF: 54 AU (24–67), PapillaCZ-20 + NaF: 11 AU (2–24), Upper dermisCZ-20 + NaF: 0 AU (0–0)) whereas FI by FM remained similar to a depth of 1000 μm ($p < 0.1$).
Conclusion: At a specific time-point, uptake of a small hydrophilic and lipophilic molecule respectively depended on CZ thickness in pretreated skin.

EARLY CAREER: *CLINICAL APPLICATIONS OF LASER & LIGHT*

CLINICAL EVALUATION OF A 1064 nm PICOSECOND LASER FOR REMOVAL OF BLACK TATTOOS IN PATIENTS WITH DARK SKIN TYPES

Hana Jeon, Daniel A. Belkin, Georgina M. Ferzli, Roy G. Geronemus
Laser & Skin Surgery Center of New York, NY

Background: Laser tattoo removal in patients with dark skin types presents a unique challenge in which there is an increased risk of scarring and dyspigmentation following each treatment. We designed a prospective study to evaluate the safety and efficacy of a 1064 nm picosecond laser for tattoo removal in patients with dark skin, and present our one year experience for this successful and ongoing study.
Study Design/Materials and Method: Seventeen patients (14 females, 3 males) between 18 and 65 years old who had Fitzpatrick skin types 5 or 6 were recruited for removal of black tattoos. 25 tattoos were enrolled. Patients were given the option of topical or injectable anesthesia prior to the procedure. Patients were offered up to ten treatments every 4–12 weeks. A 1064 nm picosecond laser (Cynosure) was used, with the settings of 1.18–2.6 J/cm^2, 2.7–4 mm spot size, 700 s pulse width, and 5 Hz. High resolution photographs were taken at each visit, and assessments for clearance, investigator satisfaction, and subject satisfaction were performed. Any side effects during or after each treatment were recorded.
Results: Fifteen patients remained in the study and 23 tattoos have been treated and followed (11 skin type 5 and 12 skin type 6). The mean age of the patients was 37 years (range 26 to 54). The mean age of the tattoos was 12 years (range 5 to 20). To date, 115 treatments have been performed. All tattoos that received 6 or more treatments showed a clearance of 50% or greater, and both the patient and investigators have been very satisfied with the treatments. Patients tolerated the treatment well, with 4 of the 15 patients (6 of 23 tattoos) tolerating the treatments without any anesthesia. The average pain scores for those who received anesthesia and for those who did not receive anesthesia were 0 and 5, respectively (0 to 10 scale).
Post treatment reactions were itching (30%), erythema (25%), and oedema (18%). Post treatment wound care was typically not required. One patient experienced hypopigmentation and there were no cases of scarring or textural change.
Conclusion: A 1064 nm picosecond laser is a safe and effective treatment for black tattoo removal in skin types 5 and 6 with minimal anesthesia and post treatment requirements.

DUAL CYCLES OF 1060 nm DIODE LASER FOR SUBMENTAL FAT

Laura Schilling, Robert A. Weiss
Maryland Laser, Skin & Vein Institute, Baltimore, MD

Background: Use of contact 1060 nm diode laser has been presented for various areas of body fat. This study was conducted using a modified device with a cycle time and cooling time modifications in order to determine optimal treatment for submental fat.
Study Design/Materials and Method: Twenty patients were treated with a novel contact cooling and 1060 nm emitting diode device strapped to the submental area of the neck. A modified 25 minute cycle was used. Images were obtained with a new method of neck photography which allows uniform head positioning. The patients wore glasses which projected 532 nm diode laser onto a target image. Patients were treated with 2 overlapping cycles at 2 visits 6 weeks apart.
Results: Results showed 75% reduction of submental fat in 15/20 patients as measured by clinical photography scale. One was a non-responder. No adverse events were noted. Erythema following treatment lasted for less than one hour. Pain score was mild to moderate with 40% of patients responding moderate.
Conclusion: The 1060 nm diode laser is safe and effective for significant reduction of submental fat. Dual cycles are very effective and give faster and more complete results.

EVALUATION OF THE EFFICACY AND SAFETY OF IPL USING A KTP FILTER FOR THE TREATMENT OF SOLAR LENTIGINES

Daniel P. Friedmann, Jennifer D. Peterson
Westlake Dermatology Clinical Research Center, Westlake Dermatology & Cosmetic Surgery, Austin, TX; Suzanne Bruce and Associates, Katy, TX

Background: Both intense pulsed light (IPL) devices and KTP lasers achieve reduction of pigmented lesions. An innovative IPL narrowband "KTP-like" filter (525-585 nm) has been developed for use in dermatology. This filter combines the benefits of the well-tolerated IPL with the precision of the KTP laser, for improved pigmentation clearance. The aim of this study was to evaluate the impact of this filter for the treatment of solar lentigines.

Study Design/Materials and Method: The study was a single center, prospective, open-label study with a before-and-after study design. The study included 15 patients with Fitzpatrick skin types II–III, presenting with flat and benign facial pigmentation. A total of three light treatments (T1-T3) were conducted at monthly intervals with an IPL system (Lumenis Ltd.) equipped with a KTP filter (525–585 nm). Three follow-up visits (FU1-FU3) were performed at 1, 3 and 6 months following the last treatment session. Pigmentation improvement was assessed by the investigator and the subject using a 5-point pigment improvement scale (0 = no improvement, 1 = trace to mild improvement of some lesions, 2 = moderate response: some lesions lighter, 3 = good response: most lesions much lighter, 4 = excellent response: most or all lesions much lighter or gone), while pigmentation clearance was assessed by the investigator using a 5-point pigmentation clearance scale. Comprehensive facial photographs with and without polarizing filter were obtained. The level of the subject's discomfort was documented using a 0–10 visual analog scale (VAS), and subject's downtime was recorded. Subject's satisfaction was noted and a personal impression questionnaire was completed. Safety was monitored throughout the study duration.

Results: Mean facial pigmentation improvement scores were 2.8, 2.9 and 3.4 at T2, T3 and FU1, respectively ($p < 0.0001$). These results represent significant improvement of facial pigmentation and also meet the study's acceptance criterion. Therefore, the study was deemed successful. Pigmentation clearance assessed by the investigator at FU1 was fair, good or excellent for 91% of treated lesions. Seventy-five percent of the subjects were satisfied or very satisfied with the treatment at FU1, with mean social downtime of 1.4, 1.5 and 0.7 days following T1, T2 and T3, respectively. Fifty percent of subjects assessed their facial pigmentation clearance as good to excellent, meaning most or all lesions were much lighter or gone, at FU1. Mean facial VAS scores were reported to be 2.2, 2.3 and 1.7 for T1, T2 and T3, respectively.

Conclusion: The use of the IPL KTP filter produced a significant improvement of solar lentigines. The state-of-the-art combination of IPL technology with a narrowband filter can provide an effective alternative to KTP lasers and an innovative approach to the available wide-spectrum IPL technology.

LONG-PULSED DYE LASER WITH MODIFIED DOUBLE-PULSING TECHNIQUE AND COMPRESSION FOR THE TREATMENT EPIDERMAL PIGMENTED LESIONS: A CASE SERIES

Jessica Gandy Labadie, Aleksander L. Krunic
Presence St. Joseph Hospital, Chicago, IL; Northwestern University, Chicago, IL

Background: Pigmentary changes brought on by age and sun exposure have always been a cosmetic concern. Recently, long-pulsed dye lasers (PDL), a known therapy for vascular lesions, have been investigated for the treatment of epidermal pigmented lesions. Using a specialized pigmented lesion compression headpiece, blood is pushed laterally out of the laser field evacuating hemoglobin and focusing laser energy on melanin. Recent studies have demonstrated excellent responses using a single-macropulse technique at the following settings: 9–12 J/cm^2, 1.5 ms, 10 mm spot size. However, the majority of these studies report patients requiring 1–4 treatments for complete lesion resolution. Herein, we describe our experiences utilizing a modified PDL double-macropulse technique to decrease the total number of treatments needed for resolution, while maintaining its safety profile.

Study Design/Materials and Method: Thirty-six patients (27 females, 9 males; skin types I–IV) with facial pigmentary epidermal lesions including lentigo simplex, lentigo solaris, or macular seborrheic keratoses participated in this study. Each lesion received 2 PDL macropulses (fluence of 9–13 J/cm^2, 1.5 ms duration and 10 mm spot size). If needed, a second treatment was delivered 6–8 weeks later. Digital photos were taken pre-treatment and 3 months post-treatment. Photos were evaluated by two independent board-certified dermatologists for efficacy, scarring, dyschromia, and non-response.

Results: Clearing was graded as: 1 = poor (<25%); 2 = fair (25–50%); 3 = good (51–75%); and 4 = excellent (>75%). Of the 36 participants, 23 had excellent clearing, 10 with good, and 3 with fair. Only one case of post-inflammatory hyperpigmentation was reported and there were no cases of scarring, hypopigmenation or purpura.

Conclusion: Long PDL with compression continues to be a safe and effective modality for treatment of epidermal pigmentary lesions. Double-pulsing decreases the total number of treatments needed for lesion resolution, while maintaining safety and minimizing side effects. Further studies are needed to fully understand the risks and benefits associated with this novel method.

SAFETY AND EFFICACY OF 1060 nm NON-INVASIVE DIODE LASER FOR FAT REDUCTION IN PATIENTS WITH BODY MASS INDEX GREATER THAN 30

Georgina M. Ferzli, Hana Jeon, Roy G. Geronemus
Laser and Skin Surgery Center of New York, New York, NY

Background: Several non-invasive modalities exist to achieve fat reduction. Devices currently available for non-invasive fat reduction have been granted FDA clearance for individuals with body mass index (BMI) of 30 or less. This study was designed to evaluate the safety and efficacy of a 1060 nm diode laser for non-invasive lipolysis in subjects with BMI greater than 30.

Study Design/Materials and Method: This study is an IRB-approved, prospective, evaluator-blinded, multicenter study evaluating the safety and efficacy of the 1060 nm diode laser for reduction of submental fat in both non-obese (BMI less than 30) and obese (BMI greater than 30) patients. Fifty-seven patients received up to two treatments at 6-week intervals with a 1060 nm diode laser. Standardized 2D photography as well as 3D ultrasound measurements of submental adipose tissue were performed at baseline and at 12 weeks post final treatment. Three blinded, independent,

board-certified dermatologists evaluated photographs to identify post-treatment images when compared to baseline images. Subjects rated their satisfaction using a 6-point Likert scale at the 12-week follow-up visit. Adverse events were recorded throughout the study. Results were categorized into two BMI groups for data analysis: one group with BMI 30 or less, and one group with BMI greater than 30. All data sets were analyzed and compared.
Results: Fifty-five subjects completed the study. Relevant statistical analysis was used to compare incidence rate and severity of adverse events between the non-obese and obese groups. Results showed that no statistically significant difference exists in adverse event profile between the two groups. Blinded evaluators were able to identify post treatment photographs in 91% and 94% of cases in non-obese and obese groups, respectively. There were no statistically significant differences in the absolute reduction (mm) of fat thickness nor the percentage of fat reduction as measured by 3D ultrasound. Finally, there was no statistically significant difference in subject satisfaction rates as 100% of the 55 subjects were satisfied with treatment.
Conclusion: The use of a non-invasive 1060 nm diode laser is safe and effective for BMIs up to 43. BMI does not appear to affect the safety or the efficacy of the treatment.

NURSING/ALLIED HEALTH

LASER TREATMENT OF PEDIATRIC PATIENTS

Tracy Ovtcharov, Clare McGrath, Lisa Pitonyak, Hana Jeon, Georgina M. Ferzli, Roy G. Geronemus
Laser & Skin Surgery Center of New York, NY

Introduction/Overview: Vascular birthmarks can be greatly distressing to the family members and pediatric patients who become more aware of the lesions as they grow older. Many of these lesions can grow in size with time, and treating them early on can make a significant difference for the patients and their families.
Analysis: This is a review of laser treatments that can be performed for pediatric patients.
Discussion: Pediatric patients with port wine stains, hemangiomas, other vascular malformations, and scars can benefit from laser treatments. Decades of experience in our large laser center supports early intervention as it allows for minimal anesthesia and maximal results.
Conclusion: Laser treatments can be performed both safely and effectively for pediatric patients.
Patient Feedback: Pediatric patients and their families can greatly benefit from laser treatments.

LASER TREATMENT OF PERIORBITAL CONDITIONS

Clare McGrath, Lisa Pitonyak, Tracy Ovtcharov, Hana Jeon, Georgina M. Ferzli, Roy G. Geronemus
Laser & Skin Surgery Center of New York, NY

Introduction/Overview: Numerous dermatologic conditions exist in the periorbital region and can be of significant distress to the patients. Given the location, treatments of these periorbital conditions can be challenging.
Analysis: This is a review of periorbital conditions that are treated at our large laser center.
Discussion: The periorbital conditions treated include port wine stains, hemangiomas, café-au-lait macules, nevus of Ota, congenital nevus, blue veins, rhytides, xanthelasmas, syringomas, festoons, and eyeliner tattoos. Appropriate placement of corneal eye shields by an experienced laser specialist allows for the safe and successful treatment of these periorbital conditions. Using a surgical lubricant to protect the eyelashes and eyebrows further increases the safety of the treatment.
Conclusion: Many periorbital conditions can be safely treated with lasers and related technologies by experienced laser specialists.
Patient Feedback: Treating these challenging conditions successfully can be highly satisfying for both the patient and the laser specialist.

RECOGNIZING COMPLICATIONS IN LASER SURGERY

Lisa Pitonyak, Tracy Ovtcharov, Clare McGrath, Hana Jeon, Georgina M. Ferzli, Roy G. Geronemus
Laser & Skin Surgery Center of New York, NY

Background: With the increased use of lasers and related technologies in the community, the incidence of complications is also expected to increase. It is important to learn about potential complications so that they can be identified early and addressed appropriately.
Study Design/Materials and Method: This is a review of complications that can arise from laser treatments as well as from related technologies.
Results: Potential complications from laser devices include scarring, infection, hyper-pigmentation, hypo-pigmentation, ignition, and lidocaine toxicity. Body sculpting devices for fat removal can result in paradoxical hyperplasia or fatatrophy. Proper patient selection, technique, prophylaxis, wound care, and careful postoperative follow-ups are imperative in avoiding such complications.
Conclusion: Early recognition of complications from laser treatments and related technologies is essential in preventing further complications. Appropriate laser and device training as well as taking preventative measures minimize potential complications in laser surgery.

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