Protocol title:

Infrared and Broadband Light for Skin Aging

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Sciton

Primary Objectives

-To assess the effect of broadband light with skin tightening properties on the gene expression pattern of aging skin

-To assess the duration of the skin rejuvenation effects of broadband light with skin tightening properties (optional)

Background

Studies in model organisms suggest that aged cells can be functionally rejuvenated, but whether this concept applies to human skin is unclear. Recently, we applied 3'-end sequencing for expression quantification ("3-seq") to discover the gene expression program associated with human photoaging and intrinsic skin aging (collectively termed "skin aging"), and the impact of broadband light (BBL) treatment. We found significant changes in 2,265 coding and noncoding RNAs, of which 1,293 that became “rejuvenated” after BBL treatment, whereby they became more similar to their expression level in youthful skin. Rejuvenated genes (RGs) included several known key regulators of organismal longevity and their proximal long noncoding RNAs. Hence, BBL treatment can restore gene expression pattern of photo-aged and intrinsically aged human skin to resemble young skin.

However, the duration of these effects and the potential to augment these effects through increases in particular wavelengths of light have not been explored. The Sciton SkinTyte (800-1800nm) is the ideal technology to examine these questions, since this device has been used in the clinical setting to reduce cheek and submental laxity (Gold, 2010). It incorporates the broadband light technology with an emphasis on 590 nm filter to achieve these clinical results.

Study Procedures

The design of this study is a prospective, single blind study of six adult volunteers.

Human subjects and sample acquisition

This study will be conducted in accord with Declaration of Helsinki principles. After Institutional Review Board approval and informed consent is obtained, six female participants over the age of 55 years will undergo BBLST treatments to the left forearm. Inclusion criteria included Fitzpatrick skin type II or III, and a global assessment of forearm skin aging consistent with moderate or severe forearm skin aging (modified validated instrument from McKenzie *et al.,* 2011) for treated participants. Treatments will be performed on the Sciton Joule Platform using BBL in Skintyte mode with 590ST filter. On a separate part of the arm that is clearly marked, Skintyte alone will be applied. Untreated areas will also be marked. All markings will be photographed. The same investigator will perform the treatments at 4-week intervals for a total of 3 treatments. At each

treatment session, two or more passes were performed. Four weeks after the third BBL treatment, 4 mm skin biopsies will performed by Keys punch technique from the BBLST treated, ST treated and adjacent untreated skin. These specimens will be bisected and placed into either RNAlater (Ambion Cat# AM7022) or formalin solution for with H&E, von Giesen or PAS staining.

Inclusion criteria

1. *Female*
2. *Age 55 years or older (total 6)*
3. *Fitzpatrick skin type 2-3*
4. *Photo-aging at least moderate on the extensor forearms*

Exclusion criteria

1. *Unable to understand and sign informed consent form*
2. *Unable to comply with study procedures*
3. *Pregnant or lactating*
4. *Prior treatment to forearms including topical retinoid, laser treatment, photodynamic treatment, prescription topical agents x 1 month*
5. *Active skin conditions that precluding treatment including zoster, blistering skin disease, psoriasis, atopic dermatitis, abnormal scarring, skin cancer in the area of study treatment*
6. *Currently on hormone based therapy (both systemic and topical)*

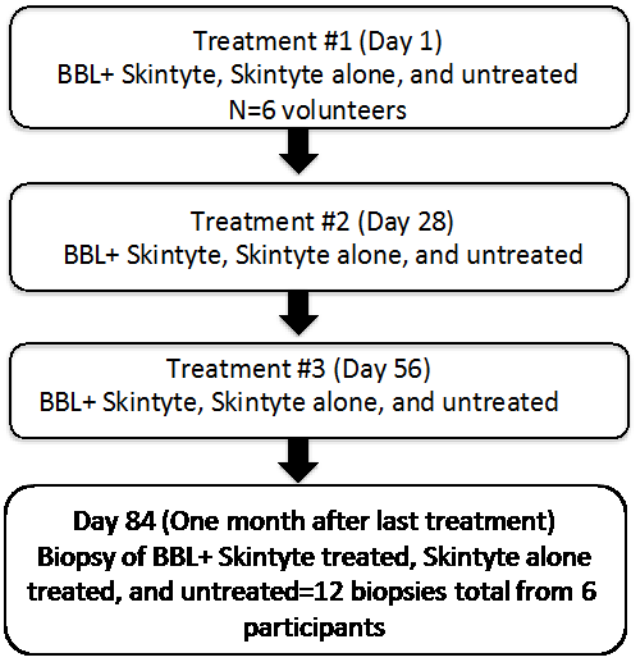
Screening visit

After written informed consent signing, subjects will undergo the following procedures, eligibility will be assessed and the following will be obtained:

- *Demographics*
- *Medical Histories*
- *Physical Examination*
- *Photographs with high resolution digital camera*
- *Cutometry*

Baseline (visit 1, treatment #1), Day 28 visit (treatment #2), Day 56 visit (treatment #3)

Each subject would have three treatments at one month intervals by the same health care provider. Each subject would have three biopsies at approximately one month post treatment (day 84 visit). The biopsies will be of the BBL treated, the BBL-ST treated and the untreated. Total biopsy per patient at the end of the study is 3.



Clinical characteristics

Mean ages, skin type and medical histories will be described. The mean elasticity and transepidermal water loss will be described for treated (day 84 and possibly 168), untreated (day 84 or day 168 (if we go with the latter budget scenario)). The degree of fine wrinkling, sagging, texture, erythema and overall appearance will be assessed using a 5-point Likert scale and compared for treated, untreated and young skin. T-tests will be used to assess the mean, standard deviations and p values at a significance level <0.05 as described in Chang *et al.,* 2013.

3-Seq and bioinformatics

Total RNA will be extracted using RNeasy Fibrous Tissue Mini Kit (Qiagen). 3-seq will be performed as described in Beck AH *et al*., 2010. In brief, oligo-dT-directed reverse transcription generated cDNAs corresponding to 3' ends of poly-adenylated transcripts; the cDNAs were cloned and subjected to deep sequencing on the Illumina GAIIx platform with raw read length 36bp. Raw reads were aligned to human genome (hg18) using bowtie (Langmead *et al*., 2009); each sample generated 6.5-12.4 million uniquely mappable reads. 3' sequencing of skin transcripts was performed to assess length distributions. Reads Per Kilobase of exon per Million mappable reads (RPKM, a direct measure of transcript abundance) and the number of raw reads falling on to each gene will be calculated using self-developed script by K. Qu. RefSeq (www.ncbi.nlm.nih.gov/RefSeq) and Ensembl (http://www.ensembl.org) annotated noncoding genes were included. Significant genes will be called using DESeq package (http://www.bioconductor.org) comparing aged-treated versus aged-untreated samples (genes changed due to treatment), and aged-untreated

versus young-untreated (genes changed due to aging). Unsupervised hierarchical clustering of significantly different expressed genes will be performed using Cluster. Treated versus untreated skin across the time points of Study Day 28 and Day 56 will analyzed. The young group will serve as the comparison group. 3'-seq technique will allow for analysis of long non-coding RNAs.

Gene Otology terms were generated using DAVID Bioinformatics Resources 6.7 (http://david.abcc.ncifcrf.gov/). Genes close to lncGenes will identified using GREAT database (http://great.stanford.edu). This data will be deposited into the Gene Expression Omnibus (GEO).

RT-qPCR

Total RNA will be extracted with TRIzol (Invitrogen) followed by RNeasy column purification (Qiagen) and DNAse Turbo Treatment (Ambion). RT-qPCR was performed using total RNA (10 ng), Taqman One Step RT-PCR master mix, and one of the following Taqman assays: GAPDH (Hs99999905_m1) and ZMPSTE24 (Hs00956778_m1) (Applied Biosystems). Reactions will be in triplicate for each sample and performed a minimum of two times. Data will be normalized to GAPDH levels.

Study Endpoints

-effect of broadband light with skin tightening properties on the gene expression pattern of aging skin as measured by 3'-end sequencing, between treated and untreated

-duration of the skin rejuvenation effects of broadband light with skin tightening properties including cutometry, transepidermal water loss, sagging, fine wrinkling, coarse wrinkling and physician global assessment between pre-treatment, day 84 (one month after last treatment) (and possibly day 156 (2 months after last treatment) if budget allows).

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