Abstract.

Photomodulation is a process which manipulates or regulates cell activity using light sources without thermal effect. Previous studies of LED Photomodulation® have shown skin textural improvement accompanied by increased collagen deposition with reduced MMP-1 (collagenase) activity in the papillary dermis. The purpose of this study was to investigate a cohort of patients (N=93) with a wide range of Fitzpatrick skin types treated by LED Photomodulation® using the Gentlewaves® full panel 590nm high energy LED array with a specific sequence of pulsing. Results showed improvement of signs of photoaging in 90%. No side effects were noted. LED Photomodulation® is a safe and effective non-painful non-ablative modality for improvement of photoaging.
Introduction

Photorejuvenation is the process whereby light energy sources are utilized to reverse the process of sun induced aging or environmental damage to the skin. Non-ablative photorejuvenation accomplishes this without disturbance of the overlying epidermis. Categories of non-ablative devices include those that affect the wound healing cascade by a thermal or photothermolysis type of injury, but we describe here a new category of non-wounding or non-thermal light based treatments which has been termed photomodulation (1).

The primary goal of non-ablative rejuvenation is induction of new collagen and dermal extracellular matrix substance which visibly improves the appearance of rhytids without disturbance or damage to the overlying epidermis. An additional goal includes the reversal of pigmented and vascular signs of photoaging which include reduction of superficial dyspigmentation (both dermal and epidermal), reduction of dermal telangiectasias and the appearance of an overall smoother texture.

Until now our thinking about how to accomplish this has involved primarily thermal mechanisms, whether it is heating of the dermis to stimulate fibroblast proliferation or heating blood vessels for photocoagulation. (2;3) Although some interest in non-thermal low-intensity laser therapy (LILT) or cold laser (very low doses of laser) has occurred, key elements required for understanding mechanisms and successful application for cell growth stimulation have been lacking. In fact red laser wavelengths have been reported as failures or with mixed results for stimulation of wound healing. (4;5)

The first experiments performed with low laser doses were revealing. McDaniel et.al. demonstrated while using various lasers at lower fluence that fibroblast activity could be regulated. (1) Using a variety of LED light sources or monochromatic laser sources, his group demonstrated that by varying fluence, procollagen synthesis could be upregulated in fibroblast culture. A clinical correlation was also shown. (6) Procollagen was measured by microarray assays. Collagen synthesis was accompanied by an equal and opposite reaction involving reduction or down regulation of matrix metalloprotease-1 (MMP-1 or collagenase). Figure 1 demonstrates results of several experiments in which procollagen is
measured by stimulation of fibroblasts in culture with a wide range of operating fluences of the pulsed dye laser. However, there is also a broad peak of collagen stimulation at fluences previously thought to be too low to produce an effect. This concept of using low energy, narrow band or coherent light with specific pulse sequences and durations has been termed photomodulation. (6) The concept that cell activity can be up or down regulated by light has been confirmed by other groups performing NASA funded research. (7;8)

Cultured fibroblasts can be stimulated by using a narrowband light emitting diode (LED) light source of various wavelengths. Based on extensive experiments by McDaniel et al beginning several years ago, LEDs emitting in the 590nm range were discovered to have the greatest effects. (1) Even more critical were results demonstrating that unless a specific sequence of pulsation was utilized, there was minimal effect on fibroblasts in culture (9). The curve in Figure 1 could be reproduced for LED by using a specific “code” of on-time and off-time (dark time). Application of continuous LED light had no effect. This mechanism of non-thermal stimulation of fibroblast growth with the specific “codes” of pulsing is now termed and patented as LED Photomodulation®.

Using many of the parameters developed in the laboratory, a multi-center clinical trial was performed on 90 patients with a series of 8 treatments over 4 weeks. (9;10) Patients were followed using stereotactic digital images, high resolution ultrasound and biopsy for Masson-trichrome, anti-collagen
and anti-MMP antibodies as well as digital profilometry. Follow-up of clinical results continued for 1 year. (11,12) This study showed very favorable results with over 90% of patients improving by at least one Fitzpatrick photoaging category and 65% of the patients demonstrating global improvement in facial texture, fine lines, background erythema and pigmentation. Results peaked at 4 – 6 months following a series of 8 treatments.

The underlying mechanism for LED Photomodulation® was determined to be stimulation of mitochondrial cell organelles with the proper “packets” of photons. This is similar to photosynthetic electron transport with a proton gradient in chloroplasts of plant cells. (13) Chloroplasts and mitochondria share very similar membrane architectures. The principle photoreceptor is chlorophyll which is cyclic tetrapyrrrole, like the heme group of cytochromes, while the molecules responsible for the absorption of light in mitochondria are the cytochrome species within the mitochondrial membrane (13)(shown in Figure 2). Both chlorophyll and cytochromes are synthesized from protoporphyrin IX. The cytochrome molecules best absorb light from 562nm to 600nm.

It is believed that light absorption causes conformation changes in antenna molecules within the mitochondrial membrane. Translocation of protons begins a pump which ultimately leads to energy for conversion of ADP to ATP, essentially recharging the cell battery and providing more energy for growth. Elegant experiments by McDaniel et al. have demonstrated the rapid production of ATP within mitochondria within cultured fibroblasts exposed to LED light only with the proper pulsing.
New ATP production occurs within seconds of LED Photomodulation® and this triggers subsequent metabolic activity of fibroblasts. (10)

This paper reports the results of an additional clinical study involving another cohort of 93 patients. They were treated by Gentlewaves® LED Photomodulation® and followed for 6 months by stereotactic digital imaging and digital microscopy. This is an additional cohort not previously reported in the 90 patient multi-center clinical trial.

Material and Methods Random patients (N=93) with mild to moderate photoaging were offered the opportunity to receive 8 LED Photomodulation® treatments over a four week period. Treatments were given with a minimum of 48 hours separation. Prior to the first treatment, the skin was cleansed with a topical masque (Gentlewaves® masque, LightBioScience, Virginia Beach, VA) Skin types ranged from Fitzpatrick type I to V. Use of OTC topicals and topical retinoids was permitted as long as no changes were made to the topical regimen within 3 months prior to the study and for the duration of the six month follow-up. All patients were coached on the daily use of sunscreen with SPF of 30 or higher. Stereotactic images were taken using the Canfield system and a Fuji S1 digital camera at day 0, 30 days, 1, 2, 3 and 6 months. The Fuji S1 CCD has 3.5 million actual photosensitive pixels on its surface, producing an effective 6 megapixel file size. Subtle changes in skin texture and other signs of photoaging are easily analyzed using high resolution baseline images for comparison.

A region just lateral to the left lateral canthus was imaged using digital microscopy using 30X and polariscopic light magnification (DG-2 Digital Microscope, Scalar America, Video Microscopes & Imaging Systems, Sacramento, CA) An acetate tracing was used with the outline of the lateral eye to accurate position the digital microscope in the follow-up visits. The position of the examined region was 2.5 cm lateral to the left lateral canthus. The resolution of the digital microscope is 330 lines at 2.3 Megapixels which allowed a detailed analysis of skin texture.

Patients performed a self-assessment of their improvement on a quartile scale and independent investigators graded images using a LCD monitor at 1280x 1064 resolution in 32 bit color mode.
Changes were graded on a quartile scale for peri-ocular wrinkles, reduction in Fitzpatrick photoaging classification, skin texture, background erythema and pigmentation.

Treatments were given with the Gentlewaves® LED Photomodulation® unit (LightBioScience, Virginia Beach, VA) with a full face panel device as shown in Figure 3. Patients were positioned 2 centimeters away from the light source. Pulsed 590nm diode light was delivered in the standard treatment regimen as previously reported. (9) Fluence range is typically from 0.1 to 0.8 Joules/cm². The pulse sequence or “code” is pre-set to the sequence yielding maximal effect in previous clinical trials and use on fibroblasts in cell culture. No user changes from pre-sets were required or permitted. All adverse events following treatment were recorded.

### Results

The Fitzpatrick scale evaluations showed a reduction of one class in over 90 percent of subjects. These results are shown in Table I.

<table>
<thead>
<tr>
<th>Photoaging Class</th>
<th>% of Subjects Reduced One Photoaging Scale Category</th>
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<tbody>
<tr>
<td>Non-responders</td>
<td>9</td>
</tr>
<tr>
<td>2 to 1</td>
<td>26</td>
</tr>
<tr>
<td>3 to 2</td>
<td>31</td>
</tr>
<tr>
<td>4 to 3</td>
<td>17</td>
</tr>
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<td>5 to 4</td>
<td>11</td>
</tr>
<tr>
<td>6 to 5</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>100 %</td>
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*Table I*
The best results clinically were seen in the category of skin texture with improvement noted by 87% of patients. (Figure 4).

Notably African-American and Asian skin types responded as well as Caucasian skin types in the textural improvement category (Figure 5).

At 6 months, independent review of images also showed reduction of peri-ocular rhytids was in 56%, reduction of background erythema in 65% and reduction of background pigmentation in 62%.

A self-assessment by patients for global improvement of skin appearance at 3 months revealed that they judged the improvement as none, mild, moderate and excellent in 8, 14, 41, 38% respectively. At 6 months the percentages fell slightly (Figure 6).
Digital microscopy in 10 patients revealed a 50 to 90% (mean textural improvement) as judged by the investigators evaluation depth of fine lines. An example of an excellent response as seen by digital microscopy is shown in Figure 7.

There were no adverse events. One patient believed that she had a flare of acne while 9 others reported an improvement in acne. Two patients noticed improvement of small patches of atopic eczema. One patient believed her eyebrows grew more thickly but this was not substantiated by the digital images. No pain or heat sensation was reported during treatment. No adverse events such as skin burning or pigmentation changes were noted in any of the subjects. Clinical results typical at 3 – 6 months following a series of 8 treatments is shown in Figure 8.
Conclusions

LED Photomodulation® performed with this specific device including this array of high energy LEDs and a very specific sequencing time code is highly effective. The initial starting regimen of 8 treatments over 4 weeks is effective for the treatment of typical signs of photoaging. The need for maintenance treatments may begin at 4 -6 months. It is unclear how often maintenance treatments will be required but our clinical experience to date suggests once a month.

It is interesting that a light source can induce similar findings for collagen synthesis and reduced MMP activity as reported with retinoic acid. (15-17) The mechanisms of a topical drug versus photomodulation are very different yet yield similar effects. It has been proposed that these effects may be synergistic, specifically with topical retinol(14). Further investigation is ongoing about these additive effects.

An anti-inflammatory effect of LED Photomodulation® was reported by one patient in the present investigation. Some preliminary data now indicates an anti-inflammatory effect for LED Photomodulation® as well. Using a solar simulator to create UV erythema, preliminary findings indicate a very noticeable reduction in UV erythema when LED Photomodulation® is supplied within hours after UV exposure. In addition low intensity light therapy with LED Photomodulation® after acute UV exposure produced significant downregulation of dermal matrix degrading enzymes which were stimulated by the UV exposure. (11)
LED Photomodulation® is painless, safe and easy to administer. It appears that this device successfully modulates the activity of fibroblasts resulting in smoother skin without the inherent risks of other thermal devices which may burn the skin or require complicated application techniques. Our results with parameters developed by fibroblast stimulation in culture appear to translate into a positive and noticeable clinical response in all skin types. Multiple trials at multiple centers are now ongoing to confirm these results. Combination treatments with standard thermal non-ablative techniques such as intense pulsed light or pulsed dye are being performed to determine whether this device may enhance results after photothermal treatments by stimulating more collagen synthesis and producing less collagen degradation.

References


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