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A prospective, randomized, placebo-controlled, double-blinded, and split-face clinical study on LED phototherapy for skin rejuvenation: Clinical, profilometric, histologic, ultrastructural, and biochemical evaluations and comparison of three different treatment settings

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Abstract

Light-emitting diodes (LEDs) are considered to be effective in skin rejuvenation. We investigated the clinical efficacy of LED phototherapy for skin rejuvenation through the comparison with three different treatment parameters and a control, and also examined the LED-induced histological, ultrastructural, and biochemical changes. Seventy-six patients with facial wrinkles were treated with quasi-monochromatic LED devices on the right half of their faces. All subjects were randomly divided into four groups treated with either 830 nm alone, 633 nm alone, a combination of 830 and 633 nm, or a sham treatment light, twice a week for four weeks. Serial photography, profilometry, and objective measurements of the skin elasticity and melanin were performed during the treatment period with a three-month follow-up period. The subject's and investigator's assessments were double-blinded. Skin specimens were evaluated for the histologic and ultrastructural changes, alteration in the status of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs), and the changes in the mRNA levels of IL-1ß, TNF-α, ICAM-1, IL-6 and connexin 43 (Cx43), by utilizing specific stains, TEM, immunohistochemistry, and real-time RT-PCR, respectively. In the results, objectively measured data showed significant reductions of wrinkles (maximum: 36%) and increases of skin elasticity (maximum: 19%) compared to baseline on the treated face in the three treatment groups. Histologically, a marked increase in the amount of collagen and elastic fibers in all treatment groups was observed. Ultrastructural examination demonstrated highly activated fibroblasts, surrounded by abundant elastic and collagen fibers. Immunohistochemistry showed an increase of TIMP-1 and 2. RT-PCR results showed the mRNA levels of IL-1ß, TNF-α, ICAM-1, and Cx43 increased after LED phototherapy whereas that of IL-6 decreased. This therapy was well-tolerated by all patients with no adverse effects. We concluded that 830 and 633 nm LED phototherapy is an effective approach for skin rejuvenation.

Keywords: Light-emitting diodes; Light therapy; Phototherapy; Photorejuvenation; Skin rejuvenation; Non-ablative rejuvenation

1. Introduction

Aging skin presents various unpleasant-looking morphologic changes such as wrinkles, dyspigmentation, telan-

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giectasia, and loss of elasticity. Both chronological and environmental influences are involved in the aging process of the skin, among which photodamage is one of the most important components [1,2]. Several characteristic histological features are observed in photodamaged skin, for example, reduction in the amount of collagen, fragmentation of collagen fibers, elastotic degeneration of elastic fibers, dilated and tortuous dermal vessels, and atrophy and disorientation of the epidermis [3,4].

So far, various rejuvenation modalities have attempted to reverse the dermal and epidermal signs of photo- and chronological aging. At the center of these treatments have been ablative methods which remove the epidermis and induce a controlled form of skin wounding to promote collagen biosynthesis and dermal matrix remodeling, such as dermabrasion, chemical peels, and ablative laser resurfacing with carbon dioxide (CO_2) or erbium: yttrium-aluminum-garnet (Er:YAG) lasers or a combination of these wavelengths [5–7]. However, these procedures require intensive post-treatment care with frequent changing of dressings, and more importantly, can lead to considerable complications including long-lasting erythema, pain, infection, bleedings, oozing, hyper- or hypopigmentation and sometimes scarring [8,9]. Patient dissatisfaction with the prolonged downtime and the clinician's desire for safer and effective rejuvenation with fewer side effects drove investigations into the development of novel skin rejuvenation procedures, leading to the appearance in the skin rejuvenation armamentarium of various nonablative rejuvenation technologies [10,11].

Nonablative skin rejuvenation aims to improve photoaged skin without destroying the epidermis [10–12]. It has been arbitrarily classified into two types, that is, type I and type II photorejuvenation [12]. The former primarily targets irregular pigmentation and telangiectasia and includes intense pulsed light (IPL) sources, 532 nm potassium-titanyl-phosphate (KTP) lasers, and high-dose 585/595 nm pulsed dye lasers (PDL), while the latter aims for wrinkle reduction and skin tightening and utilizes amongst other photothermal modalities IPL systems [13–15], low-dose 585 nm PDLs [16–21], 1064 & 1320 nm neodymium: yttrium-aluminum-garnet (Nd:YAG) lasers [22], 1450 nm diode lasers [23], and 1 540 nm erbium glass lasers [24].

The light-emitting diode (LED) is a novel light source for nonablative skin rejuvenation. It is considered to be effective for improving wrinkles and skin laxity, thus being classified under type II photorejuvenation [12,25–29]. Whereas most other techniques for type II photorejuvenation use heat energy to cause controlled thermal injury to the dermis (photothermolysis) [10–24], LED phototherapy is a non-thermal and atraumatic treatment which stimulates cell activities and functions through a photobiomodulative effect. Photobiomodulation is the process where the incident photons are absorbed by chromophores, for example in the respiratory chain of the mitochondria for longer wavelength visible light and in cellular membranes for near infrared light, to modulate various cell functions and is believed to result in new collagen synthesis to exert the effects leading to rejuvenation [25–36]. However, there is a lack of well-designed clinical studies performed in a randomized, controlled trial using objective methods to measure the treatment efficacy [12]. In the present study, we performed a prospective, randomized, placebo-controlled, double-blinded, and split-face clinical trial to determine the clinical efficacy of LED phototherapy for skin rejuvenation, and investigated post-phototherapy histological, ultrastructural, and biochemical changes. The clinical efficacy was assessed objectively by using profilometry and other instrumental measurements of the skin elasticity and amount of melanin. In addition, three treatment settings with different wavelengths of LEDs were compared in regards to the clinical, histologic, ultrastructural and biochemical changes after treatment.

2. Materials and methods

2.1. Patients

A total of 112 patients (2 males and 110 females), ranging in age from 35 to 55, with visible signs of aging were recruited for this study and randomly divided into four groups of 28 patients each. The number of subjects was calculated statistically (SAS version 9.1), so that this study would detect differences in the mean percentage improvements among the four different treatment groups when the maximum standardized effect size was larger than 0.5, at a 5% significance level, using an analysis of variance (ANOVA) with 80% power, allowing 10% extra for drop-outs [37]. Exclusion criteria included a history of photosensitivity or recent use of photosensitizing drugs including systemic retinoids, recent use of topical retinoic acid, recent history of any skin disease, operation, trauma, systemic disease that could affect the skin status, psychological disease, pregnancy, lactation and smoking. Patients were also excluded if they had had any other previous aesthetic procedures, such as botulinum toxin (botox) or filler injection, laser resurfacing, chemical peels, dermabrasion, or nonablative rejuvenation treatments, within the three years previous to the trial. This study was approved by our institutional review board. Written informed consent for the treatment and for the clinical photography was obtained from all study patients. Nineteen patients who volunteered to undergo biopsies gave their signed consent forms before entry to the trial.

2.2. Light source

The phototherapy system used as the light source for this study consisted of a base and interchangeable heads emitting quasimonochromatic light of each different preset wavelength from adjustable planar arrays of LEDs. The near infrared head (Omnilux plus^{TM}, Photo Therapeutics Ltd., Fazeley, UK) comprised five articulated panels containing 108 LEDs each, so that they could be adjusted to