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ORIGINAL ARTICLE

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A reasonable mechanism for visible light-induced skin rejuvenation

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Abstract In recent years, much research has been done in the field of non-ablative skin rejuvenation. This comes as a response to the continuous demand for a simple method of treating rhytides, UV exposure, and acne scars. Numerous researches involve visible light-pulsed systems (20–30 J/cm^2). The mechanism of action is believed to be a selective heat-induced denaturalization of dermal collagen that leads to subsequent reactive synthesis (Bitter Jr., Dermatol. Surg., 26:836–843, 2000; Fitzpatrick et al., Arch. Dermatol., 132:395–402, 1996; Kauvar and Geronemus, Dermatol. Clin., 15:459–467, 1997; Negishi et al., Lasers Surg. Med., 30:298–305, 2002; Goldberg and Cutler, Lasers Surg. Med., 26:196–200, 2000; Hernandez-Perez and Ibeitt, Dermatol. Surg., 28:651–655, 2002). In this study, we suggest a different mechanism for photorejuvenation based on light-induced reactive oxygen species (ROS) formation. We irradiated collagen in vitro with a broadband of visible light (400–800 nm, 24–72 J/cm^2) and used the spin trapping coupled with electron paramagnetic resonance spectroscopy to detect ROS. Irradiated collagen resulted in hydroxyl radicals formation. We propose, as a new concept, that visible light at the energy doses used for skin rejuvenation (20–30 J/cm^2) produces high amounts of ROS, which destroy old collagen fibers, encouraging the formation of new ones. On the other hand, at inner depths of the skin, where the light intensity is much weaker, low amounts of ROS are formed, which are well known to stimulate fibroblast proliferation.

Keywords ROS · Skin rejuvenation · Collagen · Visible light

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Introduction

Many studies currently examine non-ablative lasers and light systems, as they improve wrinkles and skin texture without harming the epidermis [1, 5, 6]. For example, intense broad visible light sources were found to be effective for superficial lesions as well as deeper cosmetic disorders including fine wrinkles and large pores in skin type 1–3 patients [4].

Authors agree that the improvement in the skin texture is associated with new collagen deposition [1–6].

In ablative treatments using, for example, CO_2 and long-pulsed Er:YAG lasers, it has been shown [3, 4, 6, 7] that the dermal collagen is heated and denaturalized, thus leading to a reactive dermal neocollagen formation, which tightens the skin [2, 3]. Also for the non-ablative treatments, it has been proposed that the first step leading to photorejuvenation is the thermally damaged collagen. For example, Negishi et al. [4] proved by histological evaluations that strong staining of type 1 and type 3 collagens occurs after a series of full-face treatment with intense pulsed visible light source. They have suggested [4] that the thermal damage to collagen fibers is caused by conducted heat from light selectively absorbed by structures containing melanin or oxyhemoglobin or by light absorbed by collagen fibers themselves.

As we have evidence from electron paramagnetic resonance (EPR) spectra that reactive oxygen species

(ROS) are formed in fibroblasts and collagen after a broadband visible light illumination, we would like to suggest that high amounts of ROS are formed in visible light-irradiated skin, which is responsible for the old collagen fibers' destruction, encouraging the formation of new ones.

Materials and methods

Illumination

We used a conventional source of visible light (400–800 nm), producing irradiance of 80 mW/cm^2, to illuminate collagen in the EPR cavity. The EPR cavity grid transmits 50% of the light energy to the samples, so, for example, during an exposure of 10 min, the collagen was illuminated with 24 J/cm^2.

EPR measurements

ROS have a very short half-life (from nanoseconds to milliseconds), making them very difficult to detect directly. By addition of a diamagnetic compound, a spin trap, which bounds the ROS, a long-lived free radical that is called the spin adduct, is produced and can be detected by the EPR technique [8]. In the present work, we used the EPR spin-trapping technique coupled with the spin trap 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), purchased from Sigma. DMPO is a common spin probe, which can trap radicals such as ·OH.

$$DMPO + \cdot OH \rightarrow DMPO - OH \quad (1)$$
$$(\textit{which yields a quartet in the EPR spectrum})$$

DMPO was purified in phosphate-buffered saline, pH 7.4, with activated charcoal in the dark.

DMPO (0.02 M) was added to the collagen solution (0.5 mg/ml), and then the solution was drawn by a syringe into a gas-permeable Teflon capillary (Zeus Industries, Raritan, NJ) of 0.032-in. inner diameter, 0.015-in. wall thickness, and 15-cm length. Each capillary was folded twice, inserted into a narrow quartz tube that was open at both ends, and then placed into the EPR cavity.

The EPR spectra were recorded on a Bruker ER 100D X-band spectrometer, before and after 6- and 10-min illuminations with the broadband light source. The microwave of the EPR was set at 9.67 GHz and the power at 20 mW. Modulation frequency and modulation amplitude were 100 kHz and 1 G, respectively; sweep width was 65 G. Time constant was 655 ms and measurement time was 168 s. Field set was 3,430 G and scans range 60 G.

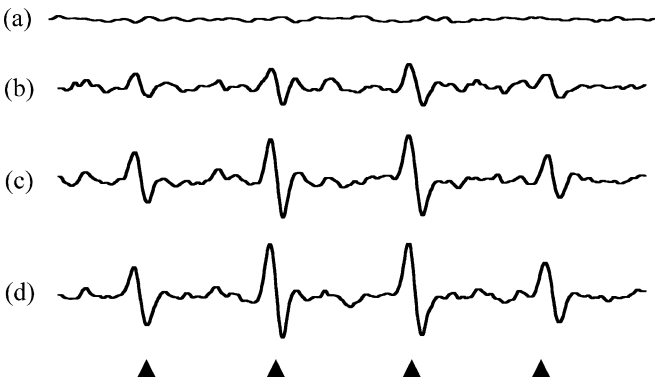

Fig. 1 EPR spectra of DMPO–OH spin adduct (assigned by *triangle*), attributed to •OH radicals formation in 0.5-mg/ml collagen illuminated with 40-mW/cm^2 broadband visible light: **a** control, **b** 10 min of illumination (24 J/cm^2), **c** 20 min of illumination (48 J/cm^2), **d** 30 min of illumination (72 J/cm^2)

Results

The EPR spectra of DMPO–OH, a quartet that monitors the existence of hydroxyl radicals [8], before and after 10, 20, and 30 min of illumination are depicted in Fig. 1b–d. Figure 1a shows baseline EPR activity (background noise). Figure 1b–d depicts the quartet indicative of hydroxyl radicals.

Discussion

It is well known [4] that visible light is absorbed by melanin and oxyhemoglobin, but as a matter of fact, every cell absorbs light in the visible range. Endogenous porphyrins, mitochondrial cytochromes, and flavoproteins [9–11] possess absorption bands in the visible and near-infrared range. Karu [12] discussed at length the possibility of cytochrome *C* oxidase as the main photoacceptor for visible light. The abovementioned chromophores are photosensitizers and generate ROS after irradiation. In Fig. 2, a scheme outlining photo-oxygenation reactions,

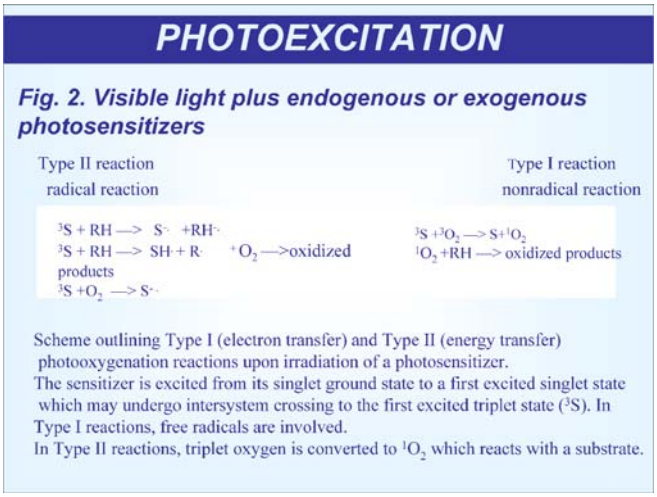

Fig. 2 Visible light plus endogenous or exogenous photosensitizers

which occur upon irradiation of endogenous photosensitizers by visible light, is shown [7].

High concentrations of ROS are known for their destructive nature. Recent experiments have shown that relatively low and controlled concentrations of ROS stimulate signal transduction processes for transcription factor activation, gene expression, muscle contraction, and cell growth [13], thus playing an important role in the activation of many cell processes. This can explain photobiostimulative effects like enhanced fibroblast proliferation [14] and wound healing [15] exerted by various low-power visible light sources.

We have already proved the formation of ROS in various cell cultures [16, 17] including fibroblasts [18–20] and attributed the enhanced proliferation of fibroblasts to low amounts of ROS generated by low-level visible light [16]. In this paper, we show that also collagen generates hydroxyl radicals upon illumination (see Fig. 1). The visible absorption spectrum of collagen can be found in the work of Negishi et al. [4].

Enhanced collagen formation after low-energy, visible-laser irradiation has been already reported by several authors [18–20].

We therefore speculate that when high concentrations of ROS are formed, they destroy old collagen fibers, encouraging the formation of new ones. But when at inner depths of the tissue, low amounts of ROS are formed; they stimulate fibroblasts and collagen metabolism, resulting in an improvement of skin texture.

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