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Differential Response of Human Dermal Fibroblast Subpopulations to Visible and Near-Infrared Light: Potential of Photobiomodulation for Addressing Cutaneous Conditions

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Background Objectives: The past decade has witnessed a rapid expansion of photobiomodulation (PBM), demonstrating encouraging results for the treatment of cutaneous disorders. Confidence in this approach, however, is impaired not only by a lack of understanding of the light-triggered molecular cascades but also by the significant inconsistency in published experimental outcomes, design of the studies and applied optical parameters. This study aimed at characterizing the response of human dermal fibroblast subpopulations to visible and near-infrared (NIR) light in an attempt to identify the optical treatment parameters with high potential to address deficits in aging skin and non-healing chronic wounds.
Materials and Methods: Primary human reticular and papillary dermal fibroblasts (DF) were isolated from the surplus of post-surgery human facial skin. An in-house developed LED-based device was used to irradiate cell cultures using six discrete wavelengths (450, 490, 550, 590, 650, and 850 nm). Light dose-response at a standard oxygen concentration (20%) at all six wavelengths was evaluated in terms of cell metabolic activity. This was followed by an analysis of the transcriptome and procollagen I production at a protein level, where cells were cultured in conditions closer to *in vivo* at 2% environmental oxygen and 2% serum. Furthermore, the production of reactive oxygen species (ROS) was accessed using real-time fluorescence confocal microscopy imaging. Here, production of ROS in the presence or absence of antioxidants, as well as the cellular localization of ROS, was evaluated.
Results: In terms of metabolic activity, consecutive irradiation with short-wavelength light ($\Leftarrow$530 nm) exerted an inhibitory effect on DF, while longer wavelengths (>=590 nm) had essentially a neutral effect. Cell behavior following treatment with 450 nm was biphasic with two distinct states: inhibitory at low- to mid- dose levels (<=30 J/cm^2), and cytotoxic at higher dose levels (>30 J/cm^2). Cell response to blue light was accompanied by a dose-dependent release of ROS that was localized in the perinuclear area close to mitochondria, which was attenuated by an antioxidant. Overall, reticular DFs exhibited a greater sensitivity to light treatment at the level of gene expression than did papillary DFs, with more genes significantly up- or down- regulated. At the intra-cellular signaling pathway level, the up- or down-regulation of vital pathways was observed only for reticular DF, after treatment with 30 J/cm^2 of blue light. At the cellular level, short visible wavelengths exerted a greater inhibitory effect on reticular DF. Several genes involved in the TGF-β signaling pathway were also affected. In addition, procollagen I production was inhibited. By contrast, 850 nm near-infrared (NIR) light (20 J/cm^2) exerted a stimulatory metabolic effect in these cells, with no detectable intracellular ROS formation. Here too, reticular DF were more responsive than papillary DF. This stimulatory effect was only observed under *in vivo*-like low oxygen conditions, corresponding to normal dermal tissue oxygen levels (approximately 2%).
Conclusion: This study highlights a differential impact of light on human skin cells with upregulation of metabolic activity with NIR light, and inhibition of pro-collagen production and proliferation in response to blue light. These findings open-up new avenues for developing therapies for different cutaneous conditions (e.g., treatment of keloids and fibrosis) or differential therapy

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