Primary human keloid fibroblasts react to Blue LED light irradiation with metabolic changes

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INTRODUCTION

Keloids are classified as a benign dermal tumour that forms as a result of abnormal skin wound healing. They are a pathological scar tissue overgrowth that expands beyond the boundary of the initial lesion. Histologically, keloids are composed by an abundance of skin connective tissue. Keloids originate from over-activity of fibroblasts cells that are involved in collagen deposition.

The major susceptible subjects are black skin people, with an equal sex distribution, commonly on chest, shoulders, earlobes, arms and cheeks. Affected individuals show often psychological disorders and motor difficulties depending on the severity of the disease.

To date, the aetiology and the mechanisms undergo keloids formations are still unclear.

METHOD AND AIM

Human keloid tissues were excised during standard aesthetic surgery and immediately underwent primary cell culture extraction. The cultures were prepared with a surgical punch in order to obtain sections of approximately 2 mm in diameter. The sections have been collected in a scratch-Petri dish keeping under standard culture conditions. Every keloids tissue has been divided in superficial derma and deep derma, in order to obtain a specific fibroblasts cell culture. When it was possible, also fibroblasts from the wound periphery have been harvested. The fibroblast cells have been seeded into appropriate support and were irradiated with blue LED light for 5, 10, 20, 30, 45 and 60 seconds. The power density of radiation has been set at 235 mW, while the irradiation was performed keeping the light source 1 cm far from cells.

The aim of this study is analyzing the effect of blue LED light irradiation on cells proliferation rate and metabolism.

To achieve this goal, we performed two colorimetric tests: WST-8 test and toxicology assay kit Sulforhodamine B based, in order to quantify the number of viable cells, proliferation and cytotoxicity compared with healthy skin fibroblasts.

We characterized the cellular type in the primary culture by immunofluorescence reveal using confocal microscopy and electrophysiological recording.

RESULTS AND DISCUSSION

The tests revealed an effect in mitochondrial activity, which could be modulated by the duration of the treatment. Fibroblasts from healthy human skin and keloid gave similar results, thus indicating an effect on a ubiquitous intracellular component. The metabolic changes also affect the cellular proliferation rate.

CONCLUSION

Blue LED light affects the metabolic activity of fibroblasts. No specific effect was found on keloid fibroblasts, thus indicating a very basic intracellular component, such as cytochromes, being the target of the treatment. This also contributes to the idea that the treatment could be safely used in keloid prone patients.

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The keloids from aesthetic surgery and sample processing

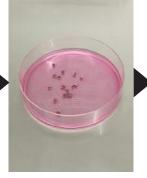




Keloid 2



Keloid after surgery

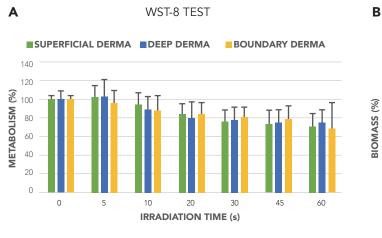


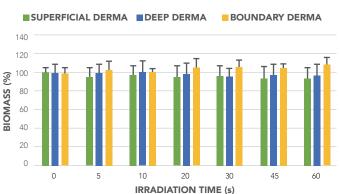
Primary culture setup



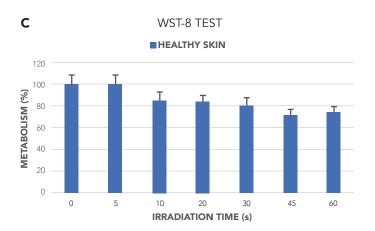
Primary culture of human fibroblasts cells

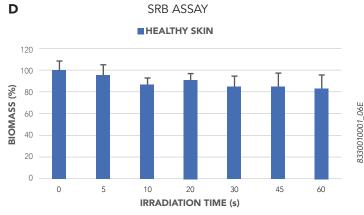
The experimental results





SRB ASSAY





The WST-8 TEST (A,C) performed on fibroblasts from keloid and healthy skin, indicates a metabolism reduction. The SRB assay (B,D) performed on the same cells shows an unchanged cell viability.