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BIOLOGICAL ACTIVITY OF 660NM RED LED IN NORMAL HUMAN ORAL KERATINOCYTE CELLS

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Phototherapy with light-emitting diodes (LEDs) has recently been attracting attention in accelerating wound healing. This study was to evaluation of cytotoxicity of normal human oral keratinocyte (NHOK) cells exposed to 660nm red LED. Results showed significantly differences between exposed and non-exposed cells in regards to cell morphology and viability

Keywords: Red LED, Human oral keratinocyte, Biological activity, cell viability, Phototherapy

1. Introduction

Light in the red to near infrared (NIR) range (630–1000 nm) generated by using low energy laser or LED arrays has been reported to have beneficial biological effects in many injury models. Red and NIR are able to pass through human tissue up to 1 inch deep. Red and near-infrared have beneficial effects on cells by "kick-starting" them into immediately creating more ATP (cellular energy) and increasing DNA and RNA activity. This effect has been carefully studied in many published reports since 1987. The positive effects occur only in injured cells. Near-infrared light via LED is a well-accepted therapeutic tool in the treatment of infected, ischemic, and hypoxic wounds and other soft tissue injuries in humans and animals [1, 2]. The purpose of this study was to evaluate NHOK cells viability when exposed to 660 nm LED light under several illumination time and intensity.

2. Material and Methods

Cell culture

NHOK cells were cultured from epithelium of retromolar pad region of patient who visited dental hospital for the extraction of 3rd molar. The connective tissue was removed by surgical scissors and put on the gauge with collagenase (0.32 mg/ml) and dispase (2.4 mg/ml) in petri dish for 60 min at 37□ incubator. The epithelial layer was detached with fine forcep from the dermis. The detached epithelium was transferred into 1 ml of the 0.05% trypsin and then minced finely using scissors. The sample was stirred with flea magnetic stirring bar for about 5-8 min at 37□ incubator. The cell was seeded onto petri dish and incubated at 37□ incubator. NHOK cells were used within 3rd passage number.

LED array illuminator

The photo illumination-cell culture system was designed to evaluate the 660 nm light on the cell viability of NHOK cells in this study (Fig. 1). Photo illumination controller is manages to adjusting of LED light intensity and illumination time. The LED module was designed to fit to the 12-well culture dish for mammalian cell culture.

Biological effects assessment

The biological effects evaluated by the irradiation with the red light were estimated at 10 min, 20 min and 30 min after exposure. Cell viability (proliferation) was assessed by MTT assay. Indeed, MTT is a direct mitochondrial activity assay, and therefore indirectly a viability assay.

3. Results and discussion

The NHOK cells have ovoid shape and grow as a monolayer. In this study, therefore, the NHOK cells were used within 3rd passage number. The cellular morphology of NHOK cells were not changed after the exposure of the 660 nm light in the condition of this study (Fig. 2). To evaluate the cell viability depend on the light energy of 660 nm wave length, MTT assay was performed (Fig. 3). In 10 min exposure condition, the cell viability of the light exposure group had increased compared with that of the control group (light non-exposure group) by 10 to 20 %. The other light exposure condition, 20 and 30 exposure tests, the cell viability of NHOK cells was almost similar to that of control group. However, the sample number is not enough to evaluate the effect of the 660 nm on the cell viability of NHOK cells, the further study is need to conform the effect of the 660 nm light. In addition, 1) the effect of higher or lower energy of 660 nm on NHOK cells compared with the condition of this study, 2) the effect of the 660 nm on gingival fibroblast for the tissue repair after injury of the gingiva, and 3) effect of the 660 nm or other wave length on the cell viability of cancer cells for the treatment of skin or mucoid epithelial tissue will be tested in the future studies.

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Fig. 1 The photo illumination-cell culture system developed in this study. (Left) photo illuminator control box; (Right) photo illuminator

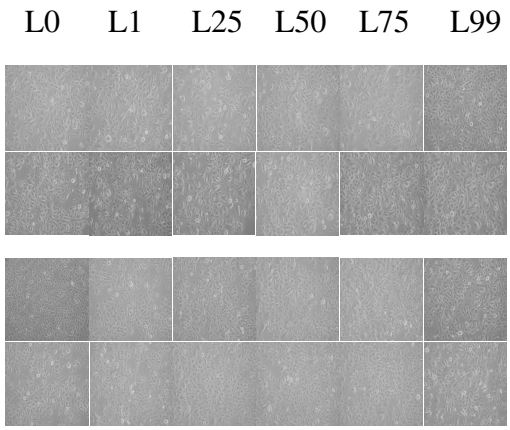

Fig. 2 The morphological changes of the normal human oral keratinocytes (NHOK) cells before and after exposure of 660 nm light for 10 (upper) and 30 (lower) min under the various light energy. (L0, 5.52 μW/cm^2; L1, 14.22 μW/cm^2; L25, 1.55 mW/cm^2; L50, 3.28 mW/cm^2; L75, 5.16 mW/cm^2; L99, 8.07 mW/cm^2).

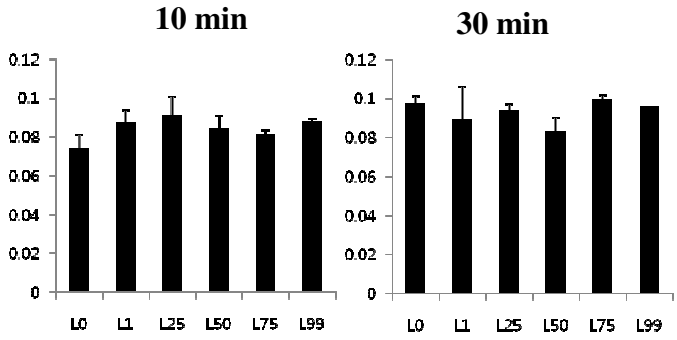

Fig. 3 The cell viability of the NHOK cells after exposure of 660 nm light for 10 and 30 min according to the amount of the light energy.