The Effects of Low Intensity Light Emitted Diode on Human Periodontal Ligament Cell and Mouse Cementoblast Cell Line

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Recommended Citation
DOI: 10.30036/TJO.201906_31(2).0001
Available at: https://www.tjo.org.tw/tjo/vol31/iss2/1

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Cover Page Footnote
This study is supported by part of the grant of the MOST 105-2314-B-040-007-MY3 and CSH-2019-C-033.

This original article is available in Taiwanese Journal of Orthodontics: https://www.tjo.org.tw/tjo/vol31/iss2/1
Objectives: The prevention of external root resorption caused by orthodontic tooth movement was endeavored by many researches. The photobiomodulation with low level dose light emitted diode (LED) is reported. The purpose of this study was to investigate the LED effects on the OCCM-30 and PDL cells proliferation.

Materials and Methods: The cementoblast cell line (OCCM-30) and periodontal ligament (PDL) cells were cultured in the FX-5000 Compression System with the 0.255 kPA compression model for one hour; the experimental groups were treated with 860 nm low LED for 20 and 60 minutes. The control group did not receive the LED treatment. The proliferation of the OCCM-30 and PDL cells were evaluated by PrestoBlue assay. The cell number were counted and statistically calculated to compare the difference.

Results: The results showed there were no morphologies changes between the control and experimental groups. The proliferation of the OCCM-30 and PDL cells showed no statistical difference between the control and experimental groups at different time interval of observation.

Conclusion: It is concluded that the 860 nm LED did not induce the cell proliferation. (Taiwanese Journal of Orthodontics. 31(2): 68-74, 2019)

Keywords: light emitted diode (LED); external root resorption; cementoblast, periodontal ligament cell (OCCM-30); cell proliferation.

INTRODUCTION

The orthodontically induced inflammatory root resorption (OIIRR) is one of most serious side effects of orthodontic treatment. It can be avoided with more cautious in orthodontic treatment procedure. The literatures had indicated that the root resorption can be classified as systematic and local risk factors. The systematic included genetics, asthma and allergies, chronic alcoholism, the severity of malocclusion, tooth-root morphology, age and gender. The local factors included the treatment duration, magnitude of applied force, direction of tooth movement, amount of apical displacement, and method of force application. It has
been considered OIIRR is a side-effect of the cellular activity associated with the removal of necrotic tissue in an overcompressed periodontal ligament (PDL). 2

The photobiomodulation therapy is defined as a form of light therapy that utilizes non-ionizing light sources, including lasers, light emitting diodes, and/or broad band light, in the visible (400 – 700 nm) and near-infrared (700 – 1100 nm) electromagnetic spectrum. The effects of photobiomodulation which exposed to low-level laser light (LLL) was proposed to have beneficial effects on enhanced tissue regeneration and tissue growth, including increase on fibroblastic and chondral proliferation, collagen synthesis, wound healing, bone regeneration, and nerve regeneration. 3–7

Light emitted diode (LED) radiation is a monochromatic near-infrared radiation (NIR) with the range of 630–1,000 nm. The LED radiation is different with the LLL radiation. The LLL is a laser with the characteristic of coherency, whereas LED light is not coherent. The LED radiation has fewer side effects and can be produced with a lower cost as compared to the LLL. 8,9 The gallium aluminum–arsenide laser with LLL was used on the experimental subject’s incisors, the result showed positive effects on bone remodeling. The findings demonstrated increased expression of fibronectin and collagen type I in the experimental group. 10

The LLL has proliferation effects on cells, it might have some repair ability on the root resorption. The aim of present study was to investigate the low 860 nm LED effects on the human PDL and mouse cementoblast cell line (OCCM-30 cells).

**MATERIALS AND METHODS**

**LED-mediated-photobiomodulation**

The LED irradiation had a wavelength of 860 nm and an optical power output of 60 mA. The radiation source was attached to a support, kept 1 cm from the culture plates. The irradiation was applied for 20 min and 60 min, administering 0.25 and 0.75 J/cm² of energy intensity.

**Cell culture**

The PDL cells and OCCM-30 cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM; Caisson Laboratories, North Logan, UT, USA) containing 10% fetal bovine serum (FBS, GeneDireX, Las Vegas, NV, USA) and 100 U/mL penicillin/100 mg/mL streptomycin (PS, Caisson Laboratories) at 37°C in a humidified atmosphere of 5% CO₂ in air. The culture medium was changed every 3 days. Cells (5x10⁴ cells/mL) were seeded on cover glasses for 24 hours, and transferred to six-well plates in a CO₂-independent medium supplemented with 10% FBS and 1% PS. Prior to cell experiments, the cover glasses were sterilized by immersion in 75% ethanol, followed by exposure to ultraviolet light for 6 hours.

**The compression and LED application**

To apply compression, the flexible plates (Cell were cultured in hydrogels in Bio Press™ Compression Plates) were subjected to static waves with various elongation (0–10%) for 1 hr. using a computer-controlled vacuum stretch apparatus (FX-5000 Compression System, FlexCell International Cooperation). Uses positive pressure to compress samples between a piston and stationary platen on the BioPress™ culture plate yielding up to 2.5kPa of force. After compression, the LED were applied on the plates and cultured for one day, three days and five days. For the control experiments, cells were cultured in the same plates with mechanical force but no LED application. At various times after the force application, cells were processed for analyses of proliferation.

**Proliferation assays**

After the various predetermined culture times, cell proliferation was evaluated using the PrestoBlue assay (Invitrogen, Grand Island, NY, USA). Briefly, at the end of the appointed time, the culture plates were harvested and washed with PBS three times. Each plate was filled with 400 mL solution (PrestoBlue: DMEM Z 1:9) and incubated at 37°C for 30 minutes. Plates were read using
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a multiwell spectrophotometer (Hitachi, Tokyo, Japan) at 570 nm, with a reference wavelength of 600 nm. The results were obtained in triplicate from three separate experiments for each test.

RESULTS

The control and experimental groups of OCCM-30 cells and PDL cell morphologies were demonstrated in Figure 1 and Figure 2. The cell shape and number of control and experimental groups were similar. The cell membrane was intact and cell morphology pattern were in the same direction.

The change in cell numbers of the control and experimental groups were shown in Figure 3 and Figure 4. There is no statistic difference between control and experimental groups. (p>0.05)

Figure 1. The morphologies of OCCM-30 cells cultured in FX-5000 Compression System under compression model observed in microscopy. The experimental groups were treated with LED, the control group was without the LED treatment.

Figure 2. The morphologies of OCCM-30 cells cultured in FX-5000 Compression System under compression model observed in microscopy. The experimental groups were treated with LED, the control group was without the LED treatment.
**Figure 3.** The quantification of the OCCM-30 and PDL cells cultured in FX-5000 Compression System under compression model and treated with LED for 20 minutes, following with the 1, 3 and 5 days.

**Figure 4.** The quantification of the OCCM-30 and PDL cells cultured in FX-5000 Compression System under compression model and treated with LED for 60 minutes, following with the 1, 3 and 5 days.
DISCUSSION

As cementum covers the outer layer of the tooth root, it bears the majority of the dynamic mechanical load during orthodontic force application and may involve or trigger in the root resorption and repair process. As the root resorption occurs, resorption lacunae can affect the cementum surface leading to root shortening during treatment. The PDL repair process initiates when cellular debris and the hyaline zone removed and newly differentiated fibroblast-like cells or epithelial cells and blood vessels migrate to the root surface.\textsuperscript{12,13}

The OCC-30 cells in the experiments was a cementoblast cell line, it promotes regeneration in the cementum structure. The PDL cell has function for regeneration, therefore it was selected in the present study. After compression mechanism applied, for physiologic repairing system the OCCM-30 should be activated. Despite the high prevalence of apical root resorption among orthodontic patients, there is no effective therapy found to date for preventing the formation of root resorption lacunae.\textsuperscript{14} The photobiostimulation cellular effects demonstrated different outcomes in various studies. Some literatures agreed with the effects but some are against it. In a systematic review of literatures showed that LLL therapy was safe concerning periodontal and root health as well as its inability to accelerate orthodontic tooth movement.\textsuperscript{15} But other article has mentioned that the LED can induce the cell proliferation.\textsuperscript{16} OrthoPulse could produce low levels of light with a near infrared wavelength of 850 nm and an intensity of 60 mW/cm\textsuperscript{2} continuous wave. Its photobiomodulation did not cause root resorption greater than the normal range that is commonly detected in orthodontic treatments.\textsuperscript{17} And there was no root resorption at all. In their experimental groups, the photobiomodulation effects has significantly increased the cell numbers. On the contrary, our study indicated that there is no difference between the control and experimental groups in cell numbers. It represented the LED was no effect on the cell growth in present study.

The optimal dose or energy density in orthodontic treatment is difficult to define. Many investigators have discovered that the biostimulation of LLL followed a dose dependency.\textsuperscript{18-19} The highest cellular activity was observed at the dose of 1.0 J/cm\textsuperscript{2}, and that energy densities of lower or higher than 1.0 J/cm\textsuperscript{2} would reduce the bio-stimulatory effect showing no statistical difference between experiment group and control groups.\textsuperscript{20} In the present study, with the light intensity lower than 1.0 J/cm\textsuperscript{2}, similar proliferation results in control and experimental group was presented. The OrthoPulse\textsuperscript{6} device which was used in the current study has a wavelength of 850 nm and a power output of 65 mW/cm\textsuperscript{2} (±13), was equivalent to 0.065 J/cm\textsuperscript{2}, which falls well below the recommended dosage for treatment efficacy.\textsuperscript{21}

In an in vivo study of rats, the 940 nm LED therapy showed improved periodontal tissue repair and decreased inflammation on root resorption after the application of orthodontic force.\textsuperscript{22} The present study, we used the wavelength of 860 nm LED on the culture cells. It would be important to know that different wavelength of the LED could cause different outcome in further investigation.

CONCLUSION

The present study indicated that the 860 nm wavelength LED could not induce the OCCM-30 or PDL cell proliferation after cultured cells were compression in laboratory condition.

ACKNOWLEDGEMENT

This study is support by part of the grant of the MOST 105-2314-B-040-007-MY3 and CSH-2019-C-033.

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