In the field of hard and neck reconstructive surgery, autologous cartilage grafting is commonly performed to reconstruct deranged nasal septum cartilage commonly used as a source of autologous graft because of its structural integrity, low immunogenicity, easy accessibility and ability to provide the bone-like support. However, in osteonecrosis, fibrosis and radiation damage, a phase of bone loss occurs and the cartilage graft becomes a clinical gift-size graft for replacing the damaged cartilage tissue. In the past 20 years, various interventions have been performed to repair damaged cartilage tissues. Clinical experiments can be summarized as monolayer cultures condition. A large number of human chondrocytes can be propagated in vitro until 15% confluence in a complete medium and at least a 95% population doubling, the chondrocyte cell yields a reproducible 25% yield of second passage cells. The source of human chondrocytes for the present study was obtained from nasal septum cartilage at the time of septoplasty surgery. In recent years, the use of low-powered laser (LPL) intervention has been reported on the nasal septum chondrocyte. Differently, we believe that the chondrocyte cell proliferation and secretion can be enhanced by modulating the local cellular chondrocyte microenvironment with a monochromatic light source. For this reason, we have conducted experiments to clarify the relationship between the effect of low-powered laser (LPL) intervention and nasal septum chondrocyte proliferation and secretion. The present study was designed to determine whether a low-powered laser (LPL) intervention stimulates nasal septum chondrocyte proliferation and secretion. These findings come to the conclusion that the irradiation with 630 nm LED can be effective in enhancing chondrocyte cell culture.

RESULTS

Cellular Proliferative Activity

On the 2nd, 8th, 15th and 22nd day after seeding, the MTT assay was performed on eight culture groups to assess cell proliferation activity. The absorbance of each sample was determined by OD 570 nm and the data was given as optical density (OD). To examine whether LED irradiation showed a significant difference in cell proliferation activity, we performed an ANOVA and post-hoc test. The results of laser irradiation showed that the OD was significantly higher than those of control groups (P<0.05) on the 8th day after irradiation. In 22nd day after irradiation, both everyday 30 min., everyday 45 min. and every other day 45 min. showed higher absorbance (P<0.05) than control groups. For evaluation of cell secretion activity, we performed type II collagen immunocytochemistry staining and Toluidine-blue metachromasia staining. The intensity of the immunostaining of irradiated cells was significantly higher than that of the control groups (P<0.05). 

Cellular Secretive Activity

As indicated in the MTT assay results, the LED irradiated chondrocytes were stained more intensely than non-irradiated chondrocytes. This procedure was performed on control and LED irradiation group. The Toluidine-blue stain was used to observe the amount of glycosaminoglycan (GAG), which was released to extracellular matrix. The toluidine-blue staining results showed that the irradiated cells were stained more intensely than the control groups (P<0.05).

DISCUSSION

Irradiation by LLLT corresponds to local application of a high-photon-density monochromatic light. LLLT effects have been considered to be induced through changes, which are influenced by cell layer, wavelength, and energy dose. These effects can be categorized into several areas: "biostimulation," "biostimulation," "cellular proliferation effect," and "inflammation suppression effect." LLLT is intended to activate cellular reactions, which are linked to the cell proliferation, cell differentiation, and collagen synthesis. LLLT is applied for various purposes, such as pain relief and so on.

The LED irradiation can be effective in stimulating nasal septum chondrocyte proliferation and secretion. These findings come to the conclusion that the irradiation with 630 nm LED can be effective in enhancing chondrocyte cell culture.

CONCLUSIONS

The study showed that a LED irradiation stimulates nasal septum chondrocytes proliferation and secretion. These findings support that the LED irradiation can be effective in enhancing chondrocyte cell culture. Moreover, for effective modulation of chondrocytes cell culture, further investigations are required. In this study, type I collagen, type II collagen, toluidine blue staining, and Toluidine-blue metachromasia were used for demonstration the bioactivity of the low-powered laser (LPL) as a result, LPL is applied for various methods. Different types of laser light have been specified for several years to get biostimulation-assisted effects. Ruby, He-Ne, Argon, CO2, and Nd:YAG lasers. The biological effects of irradiation depend on both the specificity of the light source and the energy density. Laser irradiation is usually delivered by specific laser therapy devices. These devices include the Nd:YAG laser, PDL (photodynamic therapy), CO2 laser, and Ar laser, which are widely used to treat dermatological conditions. These devices have been studied in various applications in different biological systems and clinical settings. However, the safety and efficacy of laser treatment has not been systematically analyzed in the literature. Further studies will be needed to clarify the effects of laser irradiation in several fields such as cellular immunity, bone repair or tissue regeneration, and anti-inflammatory effects.

KEY WORDS : LED, chondrocyte, cell culture, low-powered laser, nasal septum, cell proliferation, and secretion.