Effects of Low-level Light Therapy at 740 nm on Dry Eye Disease *In Vivo*

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Received October 23, 2019
Accepted November 26, 2019

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Background and Objectives

Low-level light therapy (LLLT) is an application of low-power light for various purposes such as promoting tissue repair, reducing inflammation, causing analgesia, etc. A previous study suggested the effect of light emitting diode (LED) light with the wavelength of 740 nm for promoting wound healing of corneal epithelial cells. This current study aimed to confirm the effect of LLLT for managing inflammation of a dry eye disease (DED) mouse model.

Materials and Methods

A total of 50C57BL/6 female mice were randomly grouped into 5 groups to compare the effect of LLLT: 1) Control group, 2) Only LLLT group, 3) Dry eye group, 4) LLLT in dry eye group, and 5) Early treatment group. DED was induced with 4 daily injections of scopolamine hydrobromide and desiccation stress for 17 days, and LLLT at 740 nm was conducted once every 3 days. To analyze the effect of LLLT on the DED mouse model, tear volume, corneal surface irregularities, and fluorescence in stained cores were measured, and the level of inflammation was assessed with immunohistochemistry.

Results

The DED mouse model showed significant deterioration in the overall eye condition. After LLLT, the amount of tear volume was increased, and corneal surface irregularities were restored. Also, the number of neutrophils and the level of inflammatory cytokines significantly decreased as well.

Conclusion

This study showed that LLLT at 740 nm was effective in controlling the corneal conditions and the degree of inflammation in DED. Such findings may suggest therapeutic effects of LLLT at 740 nm on DED.

Key words

Low-level light therapy; Dry eye disease; Mouse model; 740 nm

INTRODUCTION

Dry eye disease (DED) is one of the most common ocular diseases in modern society. It is a chronic multifactorial condition on the ocular surface, which has been estimated to be prevalent between 5% and > 30% in various age groups worldwide, including Korean elderly population.^{1,2}

DED is characterized by various symptoms such as dryness, irritation, blurred vision, light sensitivity, irregularity of corneal surface, etc.³⁻⁶ Although the pathogenesis has not been fully understood yet, there are many known causes of DED including hormone imbalance, tear film hyperosmolarity, meibomian gland dysfunction (MGD), and inflammatory damages.^{7,8}

Among the known pathogenesis, inflammation in particular is thought to be the major cause of DED. When there are damages on the ocular surface and tear film, a chain inflammation reaction begins in a way that inflammatory cytokines are generated from the activated inflammatory cells. This may result in destruction of lacrimal glands and impairment of conjunctival epithelium.^{9,10}

Considering that controlling the inflammation is crucial in treating patients, many options have been chosen to accomplish the goal. Topical agents are thought to be the most effective so far, but some steroid reagents are known to have side effects when they are used for a long period such as elevation of intraocular pressure, cataract formation, infection, etc.¹¹

Low-level light (laser) therapy (LLLT) has been widely used recently not only to reduce inflammation but also to relieve pain, without any complications. LLLT is non-invasive, non-ablative and only requires a short period of time to treat the symptoms. It is effective in promoting contraction of untreated wounds, suggesting an indirect effect on surround tissues.^{12,13}

Currently there are a number of studies reported re-

garding the use of low-level light on different eye conditions, but no study confirmed the effect of near infrared light (NIR) at 740 nm on eye inflammation *in vivo*. Therefore in this study, we aimed to observe and confirm the effect of LLLT on the inflammation of a dry eye mouse model.

MATERIALS AND METHODS

Dry eye mouse model

This research protocol was approved by the Dankook University Medical School Research Institutional Animal Care and Use Committee (DKU-17-023). All animals were treated in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. The study used 6-8-week-old C57BL-6 female mice (Fig. 1). A total of 50 mice were randomly assigned to five equal groups according to LLLT treatment as follows: (1) untreated control mice that were not exposed to desiccating stress or treated topically (Control group (Dry-, LED-)); (2) LLLT mice that were not exposed to desiccating stress, but only treated with LLLT (Only LLLT group (Dry-, LED+)); (3) dry eye mice (Dry eye group (Dry+, LED-)); (4) dry eye mice treated with LLLT (LLLT in dry eye group (Dry+, LED+)); and (5) early treatment of dry eye with LLLT (Early treatment group).

DED was induced and maintained in the Dry eye group, LLLT in dry eye group, and Early treatment group by subcutaneous injection of 2.5 mg/mL scopolamine hydrobromide (Sigma-Aldrich, St. Louis, MO, USA) four times (9 a.m., 12 p.m., 3 p.m., and 6 p.m.) a day and desiccation stress with air draft using fans for 18 hours (3 p.m. to 9 a.m.) daily for 17 days.^{14,15}

Low-level light therapy treatment

LLLT group and LLLT in dry eye group were treated

Fig. 1. Experimental protocols. DED was induced by scopolamine hydrobromide injection and desiccation stress in Dry eye group, LLLT in dry eye group, and Early treatment group. LLLT was applied on the Only LLLT group, LLLT in dry eye Group, and Early treatment Group.

with 740 nm LED irradiation every three days from day 5, a total of 5 times in 17 days. Early treatment group was treated with the same parameters every three days from day 1. The light dose measure for energy density (fluence) was 0.9 J/cm^2 with the duration of each treatment set, with a continuous wave for 40 seconds.

Tear volume measurements

The tear volume was measured in all mice using phenol red-impregnated cotton threads (Zone-Quick, Showa Yakuhin Kako. Co. Ltd, Tokyo, Japan), as previously described.¹⁶ Briefly, the cotton threads were applied to the lateral canthus for 20 seconds to measure the tear volume, which was expressed in mm of thread that turned red. A standard curve was constructed to convert distance to volume.

Corneal surface irregularities and corneal staining scores

The mice were anesthetized with a combination of tiletamine-zolazepam-xylazine (Zoletil™ 50, Virbac, TX, USA; Rompun®, Bayer, Leverkusen, Germany) for corneal surface irregularity analysis and corneal staining scores. Images of a white ring on the corneal surface were acquired using a stereoscopic zoom microscope (Nikon, Tokyo, Japan), right after 3 manual blinking. Corneal surface irregularities in mice were assessed and compared based on the number of distorted areas in the reflected ring.

Corneal fluorescence staining was performed with Fluorescein (Alcon®, TX, USA). The eyes were examined for

fluorescein staining with a slit-lamp biomicroscope. Corneal staining was evaluated using the Oxford schema.¹⁷ The severity grades of corneal staining were as follows: 0, normal; 1-2, mild to moderate; and ≥ 3 , severe.¹⁸

Histology and immunohistochemistry

On the 18th day, the lacrimal gland and cornea of the mice in all groups were excised after CO₂ euthanasia. The extracted lacrimal gland corneal tissues were fixed in 4% formalin and embedded in paraffin. The tissue was then sectioned with a microtome at a thickness of 5 μm , for hematoxylin and eosin staining (H&E) and immunohistochemistry.

For immunohistochemistry, the sections were washed twice with phosphate-buffered saline (PBS) for 5 minutes each and then incubated with normal blocking serum for 1 hour. The sections were then incubated with IL-1 β (ab9722, Abcam, Cambridge, MA), IL-6 α (ab208113, AbFrontier, Seoul, Korea), or TNF- α (ab6671, Abcam, Cambridge, MA) primary antibodies overnight at 4°C in a humid chamber, followed by washing with PBS twice for 5 minutes each, then incubated with biotinylated secondary antibody (BA-1000, Vector Laboratories Inc., Burlingame, CA, USA) for 1 hour. Sections were then treated with ABC solution (VECTASTAIN® Elite® ABC kit; Vector Laboratories Inc., Burlingame, CA, USA) for 1 hour, washed with PBS, and incubated with [3,3'-diaminobenzidine tetrahydrochloride] substrate for 2 minutes. Counterstaining was carried out using Meyer's hematoxylin (Dako, Carpinteria, CA, USA) counterstain. Stained sections were viewed with

Fig. 2. Tear volume measurements in Control group, Only LLLT group, Dry eye group, LLLT in dry eye group, and Early treatment group, on day 10 (A) and day 16 (B) after desiccation stress. The tear volume on day 16 days was increased in LLLT in dry eye group compared with that in Dry eye group. * $p < 0.05$, *** $p < 0.001$ compared with Dry eye group.

Fig. 3. The effect of low-level laser therapy (LLLT) on corneal surface irregularities on day 10 (A) and day 16 (B). The groups with LLLT treatment showed significantly recovered corneal irregularities on day 16 of desiccation stress.

Fig. 4. The effect of low-level laser therapy (LLLT) on corneal fluorescein staining. Representative photographs (A), mean corneal fluorescein staining score of the experiment groups on day 10 (B) and day 16 (C) after desiccation stress. On the day 16, the corneal fluorescein staining score was reduced in LLLT in dry eye group compared with that in Dry eye group. * $p < 0.05$, *** $p < 0.001$ compared with Dry eye group.

a fluorescence microscope (BX51, Olympus, Japan), and the processed images were analyzed for counts using ImageJ software (National Institutes of Health, Bethesda, MD, USA).^{19,20}

Statistical analysis

The data were analyzed using GraphPad Prism (GraphPad, San Diego, CA, USA) and are expressed as the mean $\pm$ standard deviation of the mean (SEM). The differences between the groups were analyzed using one-way analysis of variance (ANOVA), and statistical significance was defined as $p < 0.05$ using Tukey's test.

RESULTS

Tear volume measurements

The tear volume was significantly decreased in Dry eye and Early treatment group on 10th day after DED induction (Fig. 2). On day 17, the mean tear volume in Dry eye group ($0.0456 \pm 0.035 \mu\text{L}$) was still lower than the Control group ($0.0768 \pm 0.021 \mu\text{L}$). However in LLLT in dry eye and Early treatment group, the tear volumes were increased to $0.1083 \pm 0.028 \mu\text{L}$ and $0.081 \pm 0.024 \mu\text{L}$, respectively.

Corneal surface irregularities

When the distortions in the white ring on corneal sur-

faces were assessed and compared in all the mice, the corneal irregularities gradually recovered in Dry eye group on 17th day of desiccation stress. LLLT in Dry eye group and the Early treatment group at 17 days showed a circular shape with no distortions, which was similar to that of the Control group (Fig. 3).

Corneal staining scores

Fluorescein staining of the cornea was significantly increased to 2.11 ± 1.27 by desiccation stress in the Dry eye group. After 9 days of LLLT treatment, the corneal fluorescein scores did not have significant difference among Dry eye group (2.11 ± 1.27), LLLT in dry eye group (1.11 ± 0.33), and Early treatment group (1.36 ± 0.63). However after 16 days, LLLT in dry eye group (0.91 ± 0.57) and Early treatment group (0.93 ± 0.27) showed significant differences when compared to Dry eye group (2.12 ± 0.99) (Fig. 4).

Dry eye disease and desquamation of the corneal epithelium

The corneal sections of the mice were stained with H&E (Fig. 5A). The degree of corneal epithelial erosions was severe in Dry eye group, and recovered after LLLT treatment. The number of detached epithelial cells were $6.51 \pm 0.97/0.1 \text{ mm}^2$ in Dry eye group, while $2.25 \pm 1.89/0.1 \text{ mm}^2$ in LLLT in dry eye group ($p < 0.001$, versus control

Fig. 5. The effect of low-level laser therapy (LLLT) on the desquamation of the corneal epithelium. Corneal section images (A) were made after 17 days of the experiment. Sections were stained with hematoxylin & eosin (H&E). The arrows indicate detached corneal epithelial cells. Scale bar: 100 μm . Detached corneal epithelial cells were count and expressed as the mean $\pm$ SEM (B). *** $p < 0.001$ compared with Dry eye group.

Fig. 6. The effect of low-level laser therapy (LLLT) on the number of neutrophils in the lacrimal gland. Lacrimal gland sections (A) were made after 17 days of the experiment. Sections were stained with hematoxylin & eosin (H&E). Scale bar: 50 μ m. Neutrophils in the lacrimal gland were count and expressed as the mean $\pm$ SEM. * p < 0.05, *** p < 0.001 compared with Dry eye group.

group) (Fig. 5B).

The effect of LLLT on the lacrimal gland

With H&E staining, the neutrophil infiltration in lacrimal gland was observed in Dry eye group (Fig. 6A). On the other hand, LLLT in dry eye and Early treatment group showed reduced number of neutrophils. As shown in Fig. 6B, the number of neutrophils was $136.22 \pm 26.56/0.1 \text{ mm}^2$ in Control group, while $249.12 \pm 39.11/0.1 \text{ mm}^2$ in Dry eye group and $191.32 \pm 24.01/0.1 \text{ mm}^2$ in LLLT in dry eye group.

The effect of LLLT on inflammatory cytokine expression

Immunohistochemistry showed the level of inflammatory cytokines in the lacrimal gland. The expressions of IL-1 β , IL-6 α , and TNF- α were significantly increased by desiccation stress (Fig. 7A). The concentrations of IL-1 β , IL-6 α , and TNF- α in the lacrimal gland increased after 17 days in Dry eye group (1.096 ± 0.023 -, 1.499 ± 0.017 -, and 1.409 ± 0.048 -fold of the control, respectively) when compared with Control group. After LLLT treatments, the levels of cytokines in LLLT in the dry eye group (1.053 ± 0.008 -, 1.415 ± 0.053 -, and 1.096 ± 0.012 -fold of the control, respectively) and Early treatment group (1.029 ± 0.006 -, 1.129 ± 0.004 -, and 1.095 ± 0.001 -fold of the con-

trol, respectively) showed significant decrease compared with Dry eye group (Fig. 7B).

DISCUSSION

DED is a multifactorial disease with complex pathophysiological process, which hinders patients' quality of life through various symptoms. Numerous treatment options are currently available for DED, but the widely diverse management options can be confusing even for eye professionals,^{21,22} and some of the treatments involve many side effects which can worsen the conditions.²³ This is why it is crucial to assess the level of DED with appropriate criteria and treat the conditions in ways with minimal side effects. LLLT is one of the known methods to treat DED with no side effects reported so far, and so this study aimed to confirm the effects of LLLT in vivo on DED mouse model.²⁴

Most commonly used criteria in diagnosing DED are the measurements of tear volume, corneal surface irregularities, and corneal fluorescein scores. These can be used not only in diagnosing DED, but also in assessing the condition of patients.²⁵ LLLT in dry eye group showed significant increase of tear volume after 17 days, with the recovery of corneal surface irregularities and decrease in corneal fluorescein scores. Also, the degree of des-

Fig. 7. The effect of low-level laser therapy (LLLT) on the level of inflammatory cytokines in the lacrimal gland. Sections of the lacrimal gland were immunostained with IL-1β, IL-6α, and TNF-α (A). Scale bar: 50 μm. Quantitation of the inflammatory cytokine levels were presented as the mean ± SEM. **p* < 0.05, ***p* < 0.01, ****p* < 0.001 compared with Dry eye group.

quamation and the number of detached epithelial cells decreased after LLLT, showing the increased density and epithelial tissue stabilization. LLLT using NIR is known to increase the migration capacity of corneal epithelial cells in short time,²⁶ and enhance the wound healing process.

To assess the degree of inflammation, the number of neutrophils and the levels of inflammatory cytokines, IL-1β, IL-6α, and TNF-α, were measured. Neutrophils are found in inflammation sites through activation of various cells and chemokines, especially in non-specific inflammatory conditions. In this study, the number of neutrophils significantly increased in DED model, and decreased

after applying LLLT. Neutrophil infiltration is often observed at ocular surface, but also in lacrimal glands with inflammatory conditions.²⁷ The activation of neutrophils is a complex process involving sequential phases with different signaling mediators,²⁸ and such phases can recruit neutrophils at damaged tissues in various conditions such as autoimmune diseases like Sjogren's syndrome or even radioactive treatments.²⁹ As well with the neutrophil levels, the levels of IL-1β, IL-6α and TNF-α increased significantly in DED mouse model, and decreased after LLLT. IL-1β, IL-6α, and TNF-α are well studied inflammatory cytokines,³⁰ and numerous studies have been made to al-

leviate inflammation with controlling these cytokines.^{31,32}

Notable results in this study were the increased levels of IL-6 α and TNF- α in DED mouse model and the decreased level of TNF- α after LLLT, compared to the other cytokines. These indicate that IL-6 α and TNF- α are the major factors affecting dry eye related inflammation and LLLT may reduce inflammation through controlling the level of TNF- α in its mechanism.^{33,34} TNF- α , one of the most important pro-inflammatory cytokines, is known to be involved in various chemokine productions and the recruitment of macrophages, neutrophils, and T-cells at inflammation site to enhance inflammatory responses.³⁵ There is still a lack of studies regarding the relationship between LLLT and cytokine levels in ocular inflammation, but some studies on other tissues such as muscle and lung showed that LLLT is effective in reducing TNF- α expression and controlling the degree of inflammation.^{36,37}

Although vast amount of studies across many fields have been reported regarding the effect of LLLT, the standards in parameter settings have been all different per each study. Frequently used wavelengths in LLLT are NIRs, but some studies have suggested that the wavelengths between 700 to 780 nm are not effective.^{38,23} This range of wavelengths is known as optical window, being overlapped with the absorption spectrum of cytochrome C oxidase.²³ Yet, a previous study confirmed the effect of 740 nm in wound healing,²⁶ implying the wavelengths in the optical window may as well be effective. Although LLLT with inadequate parameters may be ineffective and even cause unwanted inhibitory effects,²² what is known regarding LLLT and its parameters is still at basic level, and more information should be needed to define accurate parameters.^{39,40}

In summary, this study confirmed the effect of LLLT at 740 nm *in vivo* on DED mouse model. The LLLT at 740 nm was considered effective in treating DED, increasing tear volume, improving corneal surface irregularities and symptom scores, alleviating inflammation through decreasing the levels of neutrophils and inflammatory cytokines such as IL-1 β , IL-6 α , and TNF- α .

ACKNOWLEDGEMENTS

This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI15C1524) and was a part of the project titled "Development of marine material based near infrared fluorophore complex and diagnostic imaging instruments",

funded by Ministry of Oceans and Fisheries, Korea.

CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

FUNDING

Supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (NRF-2019R111A3A01059102) and was a part of the project titled "Development of Marine Material Based Near Infrared Fluorophore Complex and Diagnostic Imaging Instruments", funded by Ministry of Oceans and Fisheries, Korea.

REFERENCES

1. Gayton JL. Etiology, prevalence, and treatment of dry eye disease. *Clin Ophthalmol* 2009;3:405-12.
2. Han SB, Hyon JY, Woo SJ, Lee JJ, Kim TH, Kim KW. Prevalence of dry eye disease in an elderly Korean population. *Arch Ophthalmol* 2011;129:633-8.
3. Stevenson W, Chauhan SK, Dana R. Dry eye disease: an immune-mediated ocular surface disorder. *Arch Ophthalmol* 2012;130: 90-100.
4. de Paiva CS, Lindsey JL, Pflugfelder SC. Assessing the severity of keratitis sicca with videokeratographic indices. *Ophthalmology* 2003;110:1102-9.
5. Goto E, Yagi Y, Matsumoto Y, Tsubota K. Impaired functional visual acuity of dry eye patients. *Am J Ophthalmol* 2002;133:181-6.
6. Musch DC, Sugar A, Meyer RF. Demographic and predisposing factors in corneal ulceration. *Arch Ophthalmol* 1983;101:1545-8.
7. Pflugfelder SC, Tseng SC, Sanabria O, Kell H, Garcia CG, Felix C, et al. Evaluation of subjective assessments and objective diagnostic tests for diagnosing tear-film disorders known to cause ocular irritation. *Cornea* 1998;17:38-56.
8. Phadatare SP, Momin M, Nighojkar P, Askarkar S, Singh KK. A comprehensive review on dry eye disease: diagnosis, medical management, recent developments, and future challenges. *Adv Pharm* 2015;2015:704946.
9. De Paiva CS, Corrales RM, Villarreal AL, Farley WJ, Li DQ, Stern ME, et al. Corticosteroid and doxycycline suppress MMP-9 and inflammatory cytokine expression, MAPK activation in the corneal epithelium in experimental dry eye. *Exp Eye Res* 2006;83:526-35.
10. Baudouin C. The pathology of dry eye. *Surv Ophthalmol* 2001;45 Suppl 2:S211-20.
11. Cutolo CA, Barabino S, Bonzano C, Traverso CE. The use of topical corticosteroids for treatment of dry eye syndrome. *Ocul*

- Immunol Inflamm 2019;27:266-75.
12. Hawkins D, Houreld N, Abrahamse H. Low level laser therapy (LLLT) as an effective therapeutic modality for delayed wound healing. *Ann NY Acad Sci* 2005;1056:486-93.
 13. Pontinen PJ. Low level laser therapy as a medical treatment modality: a manual for physicians, dentists, physiotherapists and veterinary surgeons. Tampere: Art Urpo Ltd; 1992.
 14. Choi JH, Kim JH, Li Z, Oh HJ, Ahn KY, Yoon KC. Efficacy of the mineral oil and hyaluronic acid mixture eye drops in murine dry eye. *Korean J Ophthalmol* 2015;29:131-7.
 15. Choi W, Lee JB, Cui L, Li Y, Li Z, Choi JS, et al. Therapeutic efficacy of topically applied antioxidant medicinal plant extracts in a mouse model of experimental dry eye. *Oxid Med Cell Longev* 2016;2016:4727415.
 16. Balboni GC, Brandi ML, Zonefrati R, Repice F. Effects of He-Ne/I. R. laser irradiation on two lines of normal human fibroblasts in vitro. *Arch Ital Anat Embriol* 1986;91:179-88.
 17. Bron AJ, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests. *Cornea* 2003;22:640-50.
 18. Labbé A, Terry O, Brasnu E, Van Went C, Baudouin C. Tear film osmolarity in patients treated for glaucoma or ocular hypertension. *Cornea* 2012;31:994-9.
 19. Brey EM, Lalani Z, Johnston C, Wong M, McIntire LV, Duke PJ, et al. Automated selection of DAB-labeled tissue for immunohistochemical quantification. *J Histochem Cytochem* 2003;51:575-84.
 20. Park IS, Chung PS, Ahn JC. Enhanced angiogenic effect of adipose-derived stromal cell spheroid with low-level light therapy in hind limb ischemia mice. *Biomaterials* 2014;35:9280-9.
 21. Xue AL, Downie LE, Ormonde SE, Craig JP. A comparison of the self-reported dry eye practices of New Zealand optometrists and ophthalmologists. *Ophthalmic Physiol Opt* 2017;37:191-201.
 22. Downie LE, Rumney N, Gad A, Keller PR, Purslow C, Vingrys AJ. Comparing self-reported optometric dry eye clinical practices in Australia and the United Kingdom: is there scope for practice improvement? *Ophthalmic Physiol Opt* 2016;36:140-51.
 23. de Freitas LF, Hamblin MR. Proposed mechanisms of photobiomodulation or low-level light therapy. *IEEE J Sel Top Quantum Electron* 2016;22:7000417.
 24. Stonecipher K, Abell TG, Chotiner B, Chotiner E, Potvin R. Combined low level light therapy and intense pulsed light therapy for the treatment of meibomian gland dysfunction. *Clin Ophthalmol* 2019;13:993-9.
 25. Findlay Q, Reid K. Dry eye disease: when to treat and when to refer. *Aust Prescr* 2018;41:160-3.
 26. Rhee YH, Cho KJ, Ahn JC, Chung PS. Effect of photobiomodulation on wound healing of the corneal epithelium through Rho-GTPase. *Med Lasers* 2017;6:67-76.
 27. Gruebbel MM. Special senses system. Nonneoplastic lesion at-
las. Research Triangle Park [Internet]. NC: National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health; 2014. [cited 2019 Jun 17]. Available at: <http://ntp.niehs.nih.gov/nnl/>.
 28. Hallett MB, Lloyds D. Neutrophil priming: the cellular signals that say 'amber' but not 'green'. *Immunol Today* 1995;16:264-8.
 29. Zhang Y, Deng C, Qian J, Zhang M, Li X. Improvement of radiotherapy-induced lacrimal gland injury by induced pluripotent stem cell-derived conditioned medium via MDK and inhibition of the p38/JNK pathway. *Int J Mol Sci* 2014;15:18407-21.
 30. Hernández-Rodríguez J, Segarra M, Vilardell C, Sánchez M, García-Martínez A, Esteban MJ, et al. Tissue production of pro-inflammatory cytokines (IL-1 β , TNF α and IL-6) correlates with the intensity of the systemic inflammatory response and with corticosteroid requirements in giant-cell arteritis. *Rheumatology (Oxford)* 2004;43:294-301.
 31. Aringer M, Smolen JS. SLE - Complex cytokine effects in a complex autoimmune disease: tumor necrosis factor in systemic lupus erythematosus. *Arthritis Res Ther* 2003;5:172-7.
 32. Djalilian AR, Nagineni CN, Mahesh SP, Smith JA, Nussenblatt RB, Hooks JJ. Inhibition of inflammatory cytokine production in human corneal cells by dexamethasone, but not cyclosporin. *Cornea* 2006;25:709-14.
 33. Choi W, Noh H, Yeo A, Jang H, Ahn HK, Song YJ, et al. The effect of TNF- α blocker HL036337 and its best concentration to inhibit dry eye inflammation. *Korean J Ophthalmol* 2016;30:302-8.
 34. Ji YW, Byun YJ, Choi W, Jeong E, Kim JS, Noh H, et al. Neutralization of ocular surface TNF- α reduces ocular surface and lacrimal gland inflammation induced by in vivo dry eye. *Invest Ophthalmol Vis Sci* 2013;54:7557-66.
 35. Arango Duque G, Descoteaux A. Macrophage cytokines: involvement in immunity and infectious diseases. *Front Immunol* 2014;5:491.
 36. Mesquita-Ferrari RA, Martins MD, Silva JA Jr, da Silva TD, Piovesan RF, Pavesi VC, et al. Effects of low-level laser therapy on expression of TNF- α and TGF- β in skeletal muscle during the repair process. *Lasers Med Sci* 2011;26:335-40.
 37. Aimbire F, Albertini R, Pacheco MT, Castro-Faria-Neto HC, Leonardo PS, Iversen W, et al. Low-level laser therapy induces dose-dependent reduction of TNF α levels in acute inflammation. *Photomed Laser Surg* 2006;24:33-7.
 38. Chung H, Dai T, Sharma SK, Huang YY, Carroll JD, Hamblin MR. The nuts and bolts of low-level laser (light) therapy. *Ann Biomed Eng* 2012;40:516-33.
 39. Hamblin MR. Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. *AIMS Biophys* 2017;4:337-61.
 40. Zein R, Selting W, Hamblin MR. Review of light parameters and photobiomodulation efficacy: dive into complexity. *J Biomed Opt* 2018;23:120901.