Biomedical Science Letters 2021, 27(2): 99~104
https://doi.org/10.15616/BSL.2021.27.2.99
eISSN : 2288-7415

Brief Communication

Anti-inflammatory Effects of Low-frequency Stimulator using Superposition of Alternating Microcurrent Wave in the Animal Models

Yoo-Jeong Kim[1,*], Seong gwang Lee[1,], Shin Jee Go[1,**], Suyeon An[1,**], Ye eun Kim[1,**], Ye in Kim[1,**], Kyung-Yae Hyun[2,***], Dong Shik Cho[3,****] and Go-Eun Choi[1,†,***]**

[1]*Department of Clinical Laboratory Science, Catholic University of Pusan, Busan 46252, Korea*
[2]*Department of Clinical Laboratory Science, Dong-Eui University, Busan 47340, Korea*
[3]*Natural Well Tech. Co. Ltd, Busan 47807, Korea*

Treatment techniques that affect homeostasis by non-invasive regulation in peripheral organs will advance disease research. Here, we demonstrate a non-invasive method of conditioning within an organ using a low-frequency stimulator superposition of alternating microcurrent wave in stages. It is first applied to the inflammatory response in H3N2-infected sinusitis mice. To check the progress of the treatment, mice were sacrificed every week for 3 weeks, nasal tissue was removed, and the inflammatory response was investigated through H & E staining. The low-frequency stimulation treatment group was found to alleviate the proliferation of epithelial cells and invasion of inflammatory cells compared to the control group as the passage of treatment time. The reduction of inflammatory cytokines in the nasal lavage fluid was observed in H3N2-infected sinusitis mice treated with of low-frequency stimulation using superposition of alternating microcurrent wave compared to H3N2-infected sinusitis mice after 3 weeks. These data demonstrate that low-frequency stimulation device in the form of using alternating current wave superposition on within organs provides a new method to regulate specific physiological functions. Therefore, it is necessary to prove the inhibitory effect of low-frequency stimulation using alternating current wave superposition on inflammatory diseases by various methods through further studies and clinical studies.

Key Words: Microcurrent, Low-frequency stimulator, Superposition of alternating microcurrent wave, Animal model, Inflammation, Influenza A (H3N2) virus

Sinusitis is an inflammation causes symptoms such as swelling and nasal mucosa or congestion (Rosenfeld et al., 2015; Seidman et al., 2015). Sinonasal inflammation affects all age groups, and women are affected more often than men (Blackwell and Villarroel, 2018). The causes of sinonasal inflammation can be variously caused by infections, air pollution and other irritants problems in the nasal cavity. Facial pain due to sinus inflammation is treated with anti-inflammatory drugs, including acetaminophen, ibuprofen, or corticosteroids (Rosenfeld et al., 2015). These drugs are expensive to treat pain and congestion, and cause problems with dependence and side effects such as irritability, insomnia, and intranasal bleeding (Ramey et al., 2006; Jin, 2015).

Tools for non-invasive disease control in peripheral organs will advance the study of disease and its effects on homeostasis and disease. Herein, we demonstrate a non-invasive

Received: June 9, 2021 / Revised: June 25, 2021 / Accepted: June 25, 2021
*Graduate student, **Ungraduate student, ***Professor, ****CEO.
†Corresponding author: Go-Eun Choi. Department of Clinical Laboratory Science, Catholic University of Pusan, Busan 46252, Korea.
Tel: +82-51-510-0563, Fax: +82-51-510-0568, e-mail: gechoi@cup.ac.kr

method of using microcurrents to regulate signal pathways in organs. In clinical practice, low-frequency therapy devices and percutaneous electrical stimulation (transcutaneous electrical nerve stimulation, TENS) electrotherapy is widely used as a means to reduce pain caused by osteoarthritis or musculoskeletal disorders or to help heal damaged tissues. Microcurrent stimulation is a treatment method that uses an electric current of less than 1,000 μA, which is hardly felt by the human body, and is distinguished from the conventional electrotherapy using mA units (Koh, 2019). According to previous studies, microcurrent stimulation is based on bioelectrical theory and cell communication theory that is affected by a specific signal transduction system between cells through intracellular ion channels, and has excellent stability and few side effects (Yi et al., 2021). In addition, since long-term use does not cause fatigue in the human body, positive effects can be expected when continuously applied in daily life (Lawson et al., 2021). When microcurrent was applied, intracellular Ca^{2+} homeostasis was regulated in patients with delayed myalgia, and pain was relieved by reducing the amount of cytokines such as interleukin-I, interleukin-6, and tumor necrosis factor-α in patients with fibromyalgia (Lambert, 2002; Oh et al., 2008). In addition, it was reported that microcurrents stimulated cell migration, proliferation, and angiogenesis in the wound site using microcurrent, and improved wound healing ability by reducing inflammatory plaque, and it was also reported to have improved effects on depression and post-traumatic memory loss (McMakin et al., 2005; Yu et al., 2014; Yennurajalingam et al., 2018). These studies show that microcurrent can be helpful for positive functions directly or indirectly on physiological activity in the body (Childs and Crismon, 1988; Park et al., 2000; Piras et al., 2021).

Therefore, in this study, in order to develop a microcurrent device for clinical application, the effect of the microcurrent emitting device on the anti-inflammatory action was confirmed using an animal model of sinusitis. The microcurrent device was provided by Natural Well Tech. Co., Ltd. (Busan, Korea). The microcurrent in the form of a stepped waveform of 7.07 mA (500 Ω) / 1.7 A / 3.4 A / 5 A and an overlap frequency of 7 Hz and 44 KHz was applied to confirm the possibility of clinical application. In this study, to investigate the anti-inflammatory effects of microcurrents, we generated data on the use and feasibility of anti-inflammatory effects with microcurrent for 4-week in sinusitis animal models induced by influenza A virus infection. The microcurrent device used was applied from the first day of virus introduction. A microcurrent device was connected with a copper plate (38×23 cm) the same size as the cage floor so that the mouse could receive electrical stimulation when the foot touched it. The voltage of the microcurrent device in the form of a step-like waveform and overlapping frequency was set to 5 V. Microcurrents were stimulated 24 hours a day for a total of 4 weeks. Human H3N2 (A/Brisbane/10/2007) was obtained from the Korea Centers for Disease Control. H3N2 was propagated in Madin-Darby Canine Kidney Cells (MDCKs; ATCC, Manassas, VA, USA) in culture media. For subsequent pNEC infections, we used an MOI 5 (4×10^5 pful/mL).

To demonstrate the effect of microcurrent against viral infection *in vivo*, the WT mice (20~23 g) were assigned randomly to three groups: a control group (n = 21 mice), a H3N2 viral infection animal group (n = 28 mice) and a group treated with H3N2 viral infection + microcurrent (n = 28 mice). A total of 21 mice (7 in each group) were randomly chosen for euthanization weekly. We were inoculated with H3N2 virus (4×10^5 pful/mL, 50 μL) or vehicle (saline) injections 3 times a week for 3 weeks. In order to observe the effect of microcurrent, microcurrent was administered daily for 3 weeks. The mice were euthanized 24 hours post-infection. C57BL/6 WT mice were purchased from Samtako Bio (Kyung Gi-Do, Korea). All tested mice were evaluated for each experiment containing 3 replicates. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Catholic University of Pusan (Approval No. CUP AEC 2020-1) and has taken steps to minimize pain in accordance with the approved guidelines. The nasal cavity was sectioned coronally and then histological analysis were performed in the sinonasal mucosa (Fig. 1). The infiltrating cells were counted in the lamina propria and then the severity of inflammation was graded (Bolger et al., 1997). Secretory hyperplasia in the epithelium also was graded (Lee et al., 2016). The severity was expressed as a scale of none (0), minimal (1), mild (2), moderate (3), and

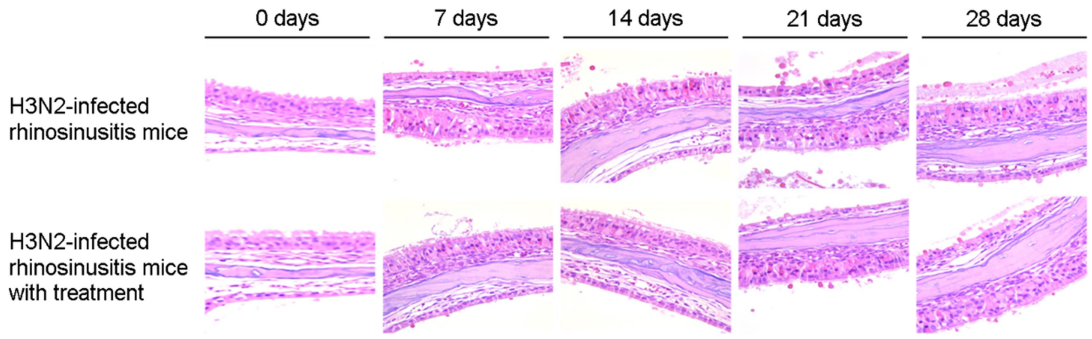

Fig. 1. Representative data of H&E staining analysis of nasal cavity in each study group. H&E staining analysis of nasal cavity in epithelium of H3N2-infected sinusitis mice treated with microcurrent (row column) and H3N2-infected sinusitis (upper column) mice. (Original magnification, ×400).

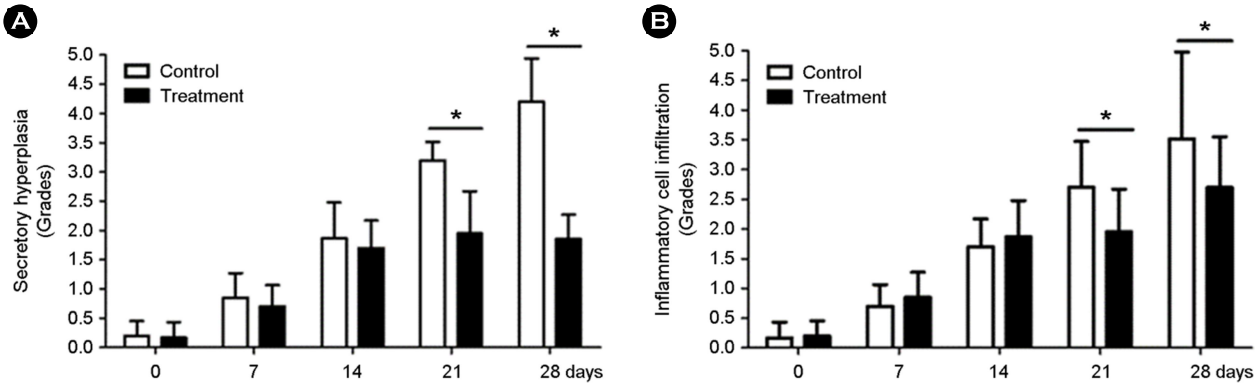

Fig. 2. Grade of inflammation for secretory hyperplasia and inflammatory cell infiltration in each study group. (A) Score of inflammation for secretory hyperplasia in each study group. H3N2-infected mice treated with microcurrent showed decreased secretory hyperplasia in the sinonasal mucosa compared with H3N2-infected mice. *P < 0.05. (B) Score of inflammatory cell infiltration in each study group. Inflammatory cell infiltrations was detected in the H3N2-infected group treated with microcurrent and compared to H3N2-infected group. *P < 0.05.

severe (4). For statistical analysis, a nonparametric Mann-Whitney U test was performed for comparison between groups using SPSS statistical software (version 16.0). P value <0.05 was considered as a significant value.

Mice infected with H3N2 showed increased secretory hyperplasia in the epithelium compared to the control group from week 2 onwards. The level of secretory hyperplasia was moderate and the distribution was multifocal. In addition, the infiltration of inflammatory cells including lymphocytes and neutrophils was increased in the H3N2 infected sinusitis mice compared to the control mice. However, the level of secretory hyperplasia in the microcurrents treatment group showed signs of alleviated hyper-proliferation in the sinus mucosa compared to the untreated group from the 3rd week onwards. The proliferation of secretory hyperplasia was mostly multifocal in both groups, but the overall stage showed a tendency to decrease from moderate to mild in the sinus mucosa compared to the untreated group. Inflammatory cells were also detected in both the microcurrents group and the untreated group. Inflammatory cell infiltration was significantly decreased in the microcurrents group compared to the untreated group from the 3rd week of treatment (Fig. 2). After the catheter was inserted into the trachea under anesthesia, 0.5 mL of physiological saline was injected into the nasal cavity and the nasal lavage fluid were collected. Interleukin (IL)-6, macrophage inflammatory protein (MIP)-2, tumor necrosis factor (TNF)-α, and interferon (INF)-γ concentrations in the nasal lavage fluid were measured using

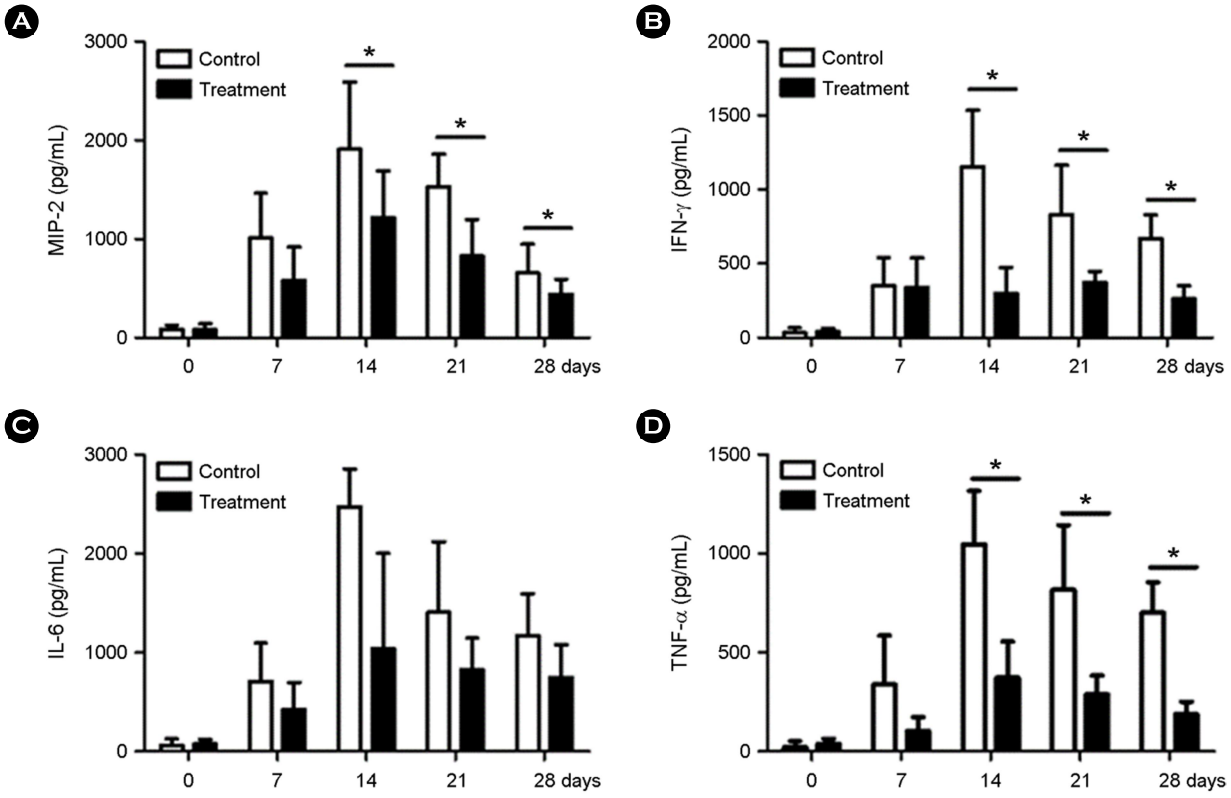

Fig. 3. Cytokine release in H3N2-infected mice treated with microcurrent and H3N2-infected mice. (A) MIP-2, (B) INF-γ: The reduction of MIP-2 and INF-γ were observed in H3N2-infected mice treated with microcurrent compared to H3N2-infected mice from the 3rd week of microcurrent treatment (*$P < 0.05$). (C) IL-6: There were no statistically significant differences between H3N2-infected group treated with microcurrent and H3N2-infected group. (D) TNF-α: TNF-α significantly decrease in H3N2-infected mice treated with microcurrent compared to H3N2-infected mice (*$P < 0.05$).

enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. Increased MIP-2, INF-γ, TNF-α production in the nasal lavage fluid was observed in H3N2-infected mice compared to controls. The reduction of MIP-2, INF-γ and TNF-α in the nasal lavage fluid was observed in H3N2-infected sinusitis mice treated with microcurrent compared to H3N2-infected sinusitis mice from the 3rd week of microcurrent treatment ($P<0.05$) (Fig. 3). Next, variable IL-6 levels were detected in H3N2-infected sinusitis mice. There were no statistically significant differences between H3N2-infected group treated with microcurrent and H3N2-infected group. Overall, these results show that H3N2-infected sinusitis mice triggers the production of proinflammatory cytokines including MIP-2, INF-γ, TNF-α and IL-6 compared to control mice. However, proinflammatory cytokines, the exception of IL-6, significantly decrease in H3N2-infected mice treated with microcurrent compared to H3N2-infected mice.

The present study measured the recovery of inflammation caused by viral infection of the sinuses during 4 weeks of microcurrent treatment. This microcurrent was found to be associated with a statistically significant decrease in epithelial cell proliferation and infiltration of inflammatory cells within the nasal epithelial tissue over time of treatment. Similarly, microcurrent has been associated with a tendency to decrease secretion of inflammatory cytokines (TNF-α, MIP-2 and IFN-γ). Importantly, this is the first report of a bio-electronic device capable of producing anti-inflammatory effects in addition to the pain-free treatment commonly associated with neuro-modulatory management of pain. The study of the tissue healing mechanisms of microcurrents has been known since the discovery of bioelectricity in tissue damage (Cheng et al., 1982). The microcurrent creates a

cell membrane potential difference through the sensitive channels of cells inside the human body, opens the cell membrane and moves Ca^{2+} ions into the cell membrane. Through chemical processes by transferred Ca^{2+} ions, ATP (adenosine triphosphate) and protein production are increased. Based on these facts, a supply of electrical energy at the cellular level that can create a cell membrane potential difference can achieve a wound healing effect 9. When tissues are damaged, the immune system affects cellular potential, and the damaged area has an overall increased resistance to the surrounding area (Becker, 1985).

This phenomenon can be interpreted as one of the causes of the appearance of an inflammatory response, and the damaged area exhibits the characteristics of the inflamed tissue, such as pain, heat, swelling, and redness, and the flow of electric current can easily pass through this body fluid. Thus, the application of microcurrents increases the intrinsic current flow in the damaged area, as it reduces the resistance of the damaged tissue, allowing the bio-current to flow more readily into the homeostasis and restore normal cellular capacity (Frick and McCauley, 2005). In this study, it was confirmed that microcurrent was applied to alleviate inflammatory cell infiltration in nasal epithelial tissue and proliferation of epithelial secretory cells caused by virus proliferation. This study is significant in that continuous non-invasive bio-electronic microcurrents use for 4 weeks reduced nasal inflammation. However, this study is limited in that the magnitude of the observed therapeutic effect has not been compared with widely used over-the-counter drugs. On the one hand, the results prove that microcurrent rarely causes minor side effects even with long-term use. These results are expected to make microcurrent safe and effective, providing an important non-drug treatment option for patients suffering from sinus congestion. A previously published sham-controlled and double-blind clinical study showed that microcurrent stimulation dramatically reduced sinus pain and that the anti-analgesic effect was significantly greater than that observed in sham-treated patients (Maul et al. 2019; Goldsobel et al., 2020). Therefore, it is necessary to prove the inhibitory effect of microcurrents (low-frequency stimulation using alternating current wave superposition) on inflammatory diseases by various methods through further studies and clinical studies.

Abbreviations

H&E, hematoxylin and eosin; IL-6, Interleukin-6; MIP-1, macrophage inflammatory protein-1; TNF-α, tumor necrosis factor-α; INF-γ, interferon-γ; ELISA, enzyme-linked immunosorbent assay.

ACKNOWLEDGEMENT

This research was supported by the Gimhae Biomedical Industry Promotion Agency & Busan Techno-Park (BTP).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Becker RO. The Body Electric. New York, William Morrow and Co. 1985.

Blackwell DL, Villarroel MA. Tables of summary health statistics for U.S. adults: 2016 National health interview survey. National Center for Health Statistics. 2018.

Bolger WE, Leonard D, Dick EJ Jr, Stierna P. Gram negative sinusitis: a bacteriologic and histologic study in rabbits. American Journal of Rhinology & Allergy. 1997. 11: 15-25.

Cheng N, Van Hoof H, Bockx E, Hoogmartens MJ, Michel J, Mulier JC, De Dijcker FJ, et al. The effects of electric currents on ATP generation, protein synthesis, and membrane transport of rat skin. Clinical Orthopaedics and Related Research. 1982. 171: 264-272.

Childs A, Crismon ML. The use of cranial electrotherapy stimulation in post-traumatic amnesia: a report of two cases. Brain Injury. 1988. 2: 243-247.

Frick A, McCauley D. Microcurrent electrical therapy. Microcurrent Electrical therapy. 2005. 25: 418-422.

Goldsobel AB, Prabhakar N, Gurfein BT. Correction to: Prospective trial examining safety and efficacy of microcurrent stimulation for the treatment of sinus pain and congestion. Bioelectronic Medicine. 2020. 6: 2.

Jin J. Nonsteroidal Anti-inflammatory Drugs. JAMA. 2015. 314: 1084.

Koh JO. Effects of Self-microcurrent massage on delayed onset muscle soreness (DOMS) and sit and reach: a preliminary

study. Journal of Sport and Leisure Studies. 2019. 73: 463-470.

Lambert MI, Marcus P, Burgess T, Noakes TD. Electro-membrane microcurrent therapy reduces signs and symptoms of muscle damage. Medicine & Science in Sports & Exercise. 2002. 34: 602-607.

Lawson D, Lee KH, Kang HB, Yang N, Llewellyn T, Takamatsu S. Efficacy of microcurrent therapy for treatment of acute knee pain: A randomized double-blinded controlled clinical trial. Clinical Rehabilitation. 2021. 35: 390-398.

Lee SB, Song JA, Choi GE, Kim HS, Jang YJ. Rhinovirus infection in murine chronic allergic sinusitis model. International Forum of Allergy & Rhinology. 2016. 6: 1131-1138.

Maul XA, Borchard NA, Hwang PH, Nayak JV. Microcurrent technology for rapid relief of sinus pain: a randomized, placebo-controlled, double-blinded clinical trial. International Forum of Allergy & Rhinology. 2019. 9: 352-356.

McMakin CR, Gregory WM, Phillips TM. Cytokine changes with microcurrent treatment of fibromyalgia associated with cervical spine trauma. Journal of Bodywork and Movement Therapies. 2005. 9: 169-176.

Oh HJ, Kim JY, Park RJ. The effects of microcurrent stimulation on recovery of function and pain in chronic low back pain. The Journal of the Korean Society of Physical Medicine. 2008. 3: 47-56.

Park RJ, Kim JS, Lee IH, Park JH, Han DU. Effects of Electrotherapy on Blood Velocity of Cranial Artery in Tension-Type Headache subjects. The Korean Society of Physical Therapy. 2000. 12: 349-359.

Piras A, Zini L, Trofè A, Campa F, Raffi M. Effects of Acute Microcurrent Electrical Stimulation on Muscle Function and Subsequent Recovery Strategy. International Journal of Environmental Research and Public Health. 2021. 18: 4597.

Ramey JT, Bailen E, Lockey RF. Rhinitis medicamentosa. Journal of Investigational Allergology and Clinical Immunology. 2006. 16: 148-155.

Rosenfeld RM, Piccirillo JF, Chandrasekhar SS, Brook I, Ashok Kumar K, Kramper M, et al. Clinical practice guideline (update): adult sinusitis. Otolaryngology-Head and Neck Surgery. 2015. 152: S1-S39.

Seidman MD, Gurgel RK, Lin SY, Schwartz SR, Baroody FM, Bonner JR, et al. Clinical practice guideline: allergic rhinitis. Otolaryngology-Head and Neck Surgery. 2015. 152: S1-43.

Yennurajalingam S, Kang DH, Hwu WJ, Padhye NS, Masino C, Dibai SS, Liu D D, Williams L, Lu Z, Bruera E. Cranial Electrotherapy Stimulation for the Management of Depression, Anxiety, Sleep Disturbance, and Pain in Patients With Advanced Cancer: A Preliminary Study. Journal of Pain and Symptom Management. 2018. 55: 198-206.

Yi D, Lim H, Yim J. Effect of microcurrent stimulation on pain, shoulder function, and grip strength in early post-operative phase after rotator cuff repair. Medicina. 2021. 57: 491.

Yu C, Hu ZQ, Peng RY. Effects and mechanisms of a microcurrent dressing on skin wound healing: a review. Military Medical Research. 2014. 1.

https://doi.org/10.15616/BSL.2021.27.2.99

Cite this article as: Kim YJ, Lee Sg, Go SJ, An S, Kim Ye, Kim Yi, Hyun KY, Cho DS, Choi GE. Anti-inflammatory Effects of Low-frequency Stimulator using Superposition of Alternating Microcurrent Wave in the Animal Models. Biomedical Science Letters. 2021. 27: 99-104.