ONLINE FIRST

ISSN: 0015-5659

e-ISSN: 1644-3284

Investigation of the effectiveness of atmospheric pressure cold plasma on sciatic nerve injury in rats

Authors: Nesibe Yılmaz, Omur Gulsum Deniz, Seyda Secgin, Yusuf Secgin, Ferhat Bozduman

DOI: 10.5603/fm.100019

Article type: Original article

Submitted: 2024-03-28

Accepted: 2024-04-25

Published online: 2024-05-13

ORIGINAL PAPER

Investigation of the effectiveness of atmospheric pressure cold plasma on sciatic nerve injury in rats

Nesibe Yilmaz[1], Omur Gulsum Deniz[2], Seyda Secgin[3], Yusuf Secgin[1], Ferhat Bozduman[4]

[1]*Faculty of Medicine, Department of Anatomy, Karabuk University, Karabuk, Türkiye*
[2]*Faculty of Medicine, Department of Histology and Embryology, Bolu Abant İzzet Baysal University, Bolu, Türkiye*
[3]*Faculty of Medicine, Department of Biochemistry, Karabuk University, Karabuk, Türkiye*
[4]*Faculty of Medicine, Department of Biophysics, Karabuk University, Karabuk, Türkiye*

*This study was supported by Karabuk University Scientific Research Projects Coordinatorship with project number KBÜBAP-23-DS-049.

Address for correspondence: Dr. Nesibe Yilmaz, Karabük University, Faculty of Medicine, Department of Anatomy, Karabuk, Turkey; e-mail: nesibeyilmaz@karabuk.edu.tr

ABSTRACT

Background: The aim of this study was to evaluate the efficacy of atmospheric pressure cold plasma jet and plasma activated medium (PAM) on sciatic nerve injury (SNI).

Materials and methods: Rats were divided into 6 groups (n = 10); group 1 (Sham), group 2 (SNI), group 3 (SNI + Atmospheric pressure cold plasma jet 5 min), group 4 (SNI + Atmospheric pressure cold plasma jet 10 min), group 5 (SNI + PAM 5 min), group 6 (SNI + PAM 10 min). On the 1st, 8th, 15th, 22nd days of the study, atmospheric pressure cold plasma jet was applied to rats in groups 3 and 4, and PAM was applied to rats in groups 5 and 6. Hot plate test was applied to all rats on the same days. On day 28, the experiment was terminated and sciatic nerve tissues were removed for histopathologic evaluations.

Results: According to the 4-week average of the hot plate tests, a significant relationship was found between group 2 and group 4 and group 6 ($p < 0.05$). When evaluated within each week, significant differences were found between group 2 and group 4 in week 1, between group 2 and group 5 and group 6 in week 2, between group 2 and group 4 in week 3, and

between group 2 and group 4 and group 6 in week 4 ($p < 0.05$). As a result of histopathologic analysis, except for the control group, the other groups had similar characteristics in terms of axonal degeneration, periaxonal swelling and axon density.

Conclusions: As a result of our study, we found that plasma application showed an improvement in the duration of the hot plate test, but did not show any improvement histopathologically.

Keywords: atmospheric pressure cold plasma jet, plasma-activated media, sciatic injury, hot plate test, histopathology

INTRODUCTION

Plasma is the fourth state of matter consisting of positive and negative ions, electrons, ultraviolet photons, neutral atoms and uncharged particles. During plasma formation, free radicals and reactive species are produced depending on the type of gas converted in it [6, 12, 20, 27, 37].

While plasma occurs naturally in the universe, it is obtained as a result of a series of processes in the laboratory. The basic principle of these processes is to obtain plasma by ionizing gases of different structures. While there are different methods for this ionization process, all of them are based on obtaining plasma by passing the gas to be used through direct or alternating current. In the medical field, dielectric barrier discharge and atmospheric pressure plasma jet methods come to the fore. Dielectric barrier discharge was introduced by Siemens in 1857. It is obtained by passing gases with different properties between a high voltage electrode made of dielectric material and a ground electrode. The part of the plasma formed by the gases ionized between the electrodes that can flow to atmospheric pressure is called atmospheric pressure plasma jet. In short, plasma jets are electrical discharge mechanisms that can leave the environment in which they are produced and move in the atmosphere. Different reactive particles such as O, OH, N2, He, etc. emerge from these plasmas produced in the laboratory environment, depending on the amount, properties and application method of the gas supplied. These particles are detected by optical emission spectroscopy [5, 14, 20, 38].

Plasma is divided into different classes as hot-cold according to the plasma temperature, full-partial according to the ionization intensity, low-atmospheric-high pressure according to the pressure 5-13-14 Hot plasmas are obtained by increasing the gas temperature

above 1000 K. Cold plasmas are obtained by lowering the gas temperature from 104 K to below 1000 K. Cold plasmas are also divided into low pressure cold plasmas and atmospheric pressure cold plasmas according to the pressure of the environment. Low-pressure cold plasma is obtained by using a pressure lower than the atmospheric pressure of 1 atm and is also called vacuum plasma. Its disadvantages include the need for more equipment and its cost. Atmospheric pressure cold plasma, on the other hand, is a class of more active species that do not need equipment such as vacuum, which is applied under 1 atm pressure and at ambient temperature [20, 36, 39].

Plasma can be applied directly or indirectly on the target tissue and plasma can show beneficial activity in both ways. Indirectly, the plasma jet is injected into water, 0.9% NaCl solution, phosphate buffered saline, organic matter solutions and the resulting medium is called plasma activated medium (PAM) [7, 30, 32].

Plasma has wide applications in many fields such as industrial, medical, textile and aircraft industries. In the medical field, cell biostimulation, wound healing, fibrin activation enhancer, tissue regeneration, surface biomodification, skin regeneration, anti-inflammatory, antimicrobial and anticancer effects are frequently utilized. Plasma has minimal effect on normal cellular activity and this effect is negligible. Therefore, it has no side effects. The cocktail of ionized gases, electrically excited particles, electrically charged particles, free electrons, reactive species in plasma stimulates angiogenesis, antimicrobial effect on biofilm, minimal temperature increase in tissue, local endogenous radicals, coagulation, wound healing and cell biostimulation by stimulating fibroblasts and keratinocytes [2, 4, 9, 15, 17, 30].

The aim of the study was to investigate the efficacy of atmospheric pressure cold plasma on sciatic nerve injury in rats.

MATERIALS AND METHODS

Ethics committee approval and animal procurement

The study was carried out with the decision of the local ethics committee of Karabük University Animal experiments dated 27/12/2022 and numbered 2022/10. The rats used in the study were obtained from Karabük University Experimental Medicine Application and Research Center. All procedures to be performed on the rats during the experiment were also performed in this center. The rats were fed ad libitum with pellet feed at 20–24 C°, 50–60% room humidity, 12 hours in light and 12 hours in darkness for 4 weeks.

Sciatic nerve injury and plasma applications

The study was performed using 60 female Wistar albino rats weighing 250–300 g. On the first day of the experiment, the gluteal and posterior thigh region of all rats were shaved under xylazine/ketamine anesthesia. In the right extremity, the biceps femoris muscle was separated and the sciatic nerve was exposed. In order to damage the sciatic nerve, the sciatic nerve was compressed with forceps 3 times with 10 s intervals and 10 s duration. In this way, crush injury was created in the sciatic nerve [40]. Rats were randomly divided into 6 groups. The groups and all procedures applied to the groups are described below;

Group 1 (Sham group); Sciatic nerves were exposed but no crush injury was created.

Group 2 (Sciatic nerve injury [SNI] group); Sciatic nerve crush injury was performed.

Group 3 (SNI + Atmospheric pressure cold plasma jet 5 min); Sciatic nerve crush injury was created and atmospheric pressure cold plasma jet was applied for 5 min for 4 weeks (28 days).

Group 4 (SNI + Atmospheric pressure cold plasma jet 10 min); Sciatic nerve crush injury was created and atmospheric pressure cold plasma jet was applied for 10 min for 4 weeks (28 days).

Group 5 (SNI + Atmospheric pressure cold plasma activated (PAM) 5 min); Sciatic nerve crush injury was created and PAM was applied for 5 min for 4 weeks (28 days).

Group 6 (SNI + Atmospheric pressure cold plasma activated (PAM) 10 min); Sciatic nerve crush injury was created and PAM was applied for 5 min for 4 weeks (28 days).

There are many different time applications in the literature about the dose of plasma, and these applications are generally 5 minutes and multiples [10, 11, 14].

Preparation of atmospheric cold pressure plasma jet and PAM

The atmospheric pressure cold plasma jet system was created using 99.99% pure argon gas, manually adjusted flow meter (3 L/min), plasma pen, high voltage electrode, oscilloscope, high voltage power supply, optical emission spectroscopy and computer (Fig. 1).

A plasma pen with a capacitive electrode design was used to generate the argon plasma. A signal of 11kV voltage and 10kHz frequency was applied to the plasma pen by the power supply to initiate the discharge. A high voltage probe and digital oscilloscope were used to measure the signal used during plasma generation (Fig. 2).

PAM was obtained by applying the plasma jet formed as a result of the system to distilled water in a petri dish for 5 min and 10 min [7, 32].

Figure 3 shows the optical emission spectrum of argon plasma. The graph was obtained using ThunderOptics brand SMA-E model 360-920nm measurement range optical

emission spectrometer. When we look at the graph, argon peaks are observed in a certain range due to argon plasma. Thanks to this graph, we have information about the homogeneous formation of the plasma.

Hot Plate test

The hot plate test is a method used to measure acute thermal pain in rats. All rats were subjected to the hot plate test on the 1st, 8th, 15th and 22nd day of the experiment. The hot plate was brought to 55 ± 1°C without placing the rats and the temperature was monitored with a digital thermometer during the test. The rats were made to stand with their hind feet on the plate. Then, the time of foot withdrawal was recorded. To prevent tissue damage, the test duration was set as maximum 15 s. If this time lasted more than 15 s, the test was terminated regardless of whether any response was observed or not and the test time was recorded as 15 s [29, 35].

Termination of the experiment and histopathological analysis

At the end of 28th day, all rats were anesthetized and decapitated. Sciatic nerve tissue samples were taken and fixed in 10% neutral formalin for 2 weeks for histopathologic analysis. Solutions were changed every 3 days. Following the completion of the 2-week fixation period, the tissues were placed in tissue tracking cassettes, labeled appropriately and kept in running water overnight and formalin was removed from the tissue. The tissues were first dehydrated by passing through alcohol series (70%, 80%, 96%, 100%). The tissues were then made transparent with xylene and infiltrated with paraplast. The tissues were removed from the hot paraffin and embedded in L-irons filled with paraffin and shaped as a square on a smooth surface. All groups were then named by placing small rectangular cardboards in the L-irons so that they did not touch the tissue, thus completing the tissue tracking process.

For light microscopic analysis, 4 µm thick sections were taken from the paraffin-embedded sections using a rotary microtome (Leica RM2125RT) with 1/100 sampling in accordance with the systematic random sampling rule. Afterwards, the sections taken from all groups were placed in a bain-marie pool with a water temperature of 42ºC and powdered gelatin for stronger adhesion of the tissues to the slide. The sections taken from the bain-marie pool were placed on slides for staining and kept in an oven at 58°C overnight to remove paraplast from the tissues and all sections were stained with hematoxylin-eosin for light microscopic analysis. Before the sections in all groups were included in the staining, a pilot study was performed on a single section to determine the penetration time of the dye into the

tissue. The slides of all groups were then stained with the relevant dye in accordance with the pilot staining procedure, covered with entellan and kept on a clean surface for a few days to dry.

Statistical analysis

For the reliability of the study, hot plate testicular results were analyzed in two separate ways according to weekly results and total results. The conformity of the data to normal distribution was analyzed by Shapiro–Wilk test. Median (minimum-maximum) values were included in the descriptive statistics of the data that did not fit the normal distribution. Kruskal Wallis H test was used to compare the groups that did not conform to normal distribution. Pairwise comprasion test was used to determine which groups were different. The data used in histopathologic analysis were expressed as Median (IQR). As a result of the normality tests of the data belonging to the groups, it was determined that the groups did not show normal distribution. Kruskal Wallis test and Mann Whitney U test with Bonferroni correction were used for comparisons of parameters between groups. Minitab 17 and Spss 21 package programs were used for statistical analysis and $p < 0.005$ was considered statistically significant.

RESULTS

Hot plate test results

When 4-week hot plate test averages were evaluated, the highest mean duration was determined in the SNI group and the lowest mean duration was determined in the sham group ($p < 0.05$). There was a decrease in the mean duration of hot plate test in the plasma jet and/or PAM treated groups compared to the SNI group (Table 1). According to the group averages obtained at the end of 4 weeks of the study, a significant difference was found between the groups by Kruskal Wallis H test ($p < 0.05$). Pairwise comprasion post hoc test showed a significant difference between 1 vs 2, 1 vs 3, 1 vs 4, 1 vs 5, 1 vs 6, 2 vs 4, 2 vs 5, 2 vs 6 groups ($p < 0.05$) (Table 1).

In the 1st, 2nd, 3rd and 4th weeks of the study, the highest hot plate test duration was recorded in the SNI group and the lowest duration was recorded in the sham group. Starting from the 3rd week, it was determined that the duration of hot plate testis in the plasma jet and/or PAM treated groups showed a significant decrease compared to the SNI group. This duration was even shorter in the group in which atmospheric cold plasma jet was applied for 10 min and in the PAM 10 min group. It was determined that the duration of atmospheric cold

plasma application rather than the type of application was more effective in the improvement in the duration of hot plate test (Table 2, Fig. 4). The groups were compared according to the weeks by Kruskal Wallis H test and a significant difference was found between the groups in all weeks ($p < 0.05$). Pairwise comprasion post hoc test was performed between the groups of 1 vs 2, 1 vs 3, 1 vs 5, 1 vs 6, 2 vs 4 in week 1, between the groups of 1 vs 2, 1 vs 3, 1 vs 4, 1 vs 5, 1 vs 6, 2 vs 5, 2 vs 6 in week 2, between the groups of 1 vs 2, 1 vs 3, 1 vs 4, 1 vs 5, 1 vs 5, 1 vs 6, 2 vs 5, 2 vs 6 in week 3. In week 3, a significant difference was found between 1 vs 2, 1 vs 3, 1 vs 5, 2 vs 4, 3 vs 4, 4 vs 5 groups, and in week 4 between 1 vs 2, 1 vs 3, 2 vs 4, 2 vs 6 groups ($p < 0.05$) (Table 2).

Histopathologic evaluation of sciatic nerve tissue

Assessment of the damage in the sciatic nerve tissue was performed using a Nikon Eclipse 80i light photomicroscope, taking into account changes such as axonal degeneration, periaxonal swelling and axonal density. Five different areas were selected and these morphologic parameters were scored semiquantitatively between 0–3. No change was considered as score 0, less than 25% change as score 1, 25–75% change as score 2, and more than 75% change as score 3. In this context, quantitative results were obtained by comparing between groups. Each parameter was performed by the same histologist without knowing which tissue sample belonged to which group and by random selection of tissue samples (Blinded evaluation).

When the relevant results were evaluated; there was a statistical difference between sham and atmospheric pressure cold plasma jet 5 min group, atmospheric pressure cold plasma jet 10 min group, sciatic injury group, SNI + PAM 5 min and SNI + PAM 10 min in terms of axonal degeneration, periaxonal swelling and axon density ($p < 0.001$). Except for the sham group, there was no statistical difference between the other groups in terms of the related parameters ($p > 0.05$) (Table 3, Fig. 5).

DISCUSSION

Plasma, which is naturally found in nature and can be obtained in the laboratory by applying high voltage to the gas, is a substance that can be used in the field of health [23]. Cold atmospheric plasma can interact with biological cells at temperatures below 40ºC and trigger various biological processes. In addition, the fact that cold atmospheric plasma application is non-contact and painless allows it to be easily used on human tissues and cells [23, 24]. Studies have shown that cold plasma has a wide range of applications. Cold

atmospheric plasma, which is used in disinfection and sterilization processes, is also a highly effective therapeutic agent in the field of health [3, 21, 25, 26]. It has important therapeutic effects in wound healing, suppression of tumor growth, various skin diseases such as psoriasis and dermatitis [10, 11, 18, 33].

Peripheral nerve injury is a pathologic condition that can occur especially after traumatic injuries. There is still no treatment method to ensure complete recovery after peripheral nerve injury. First, an injury occurs in the peripheral nerve due to trauma. This is followed by a second injury involving biochemical and metabolic processes that lead to cell dysfunction and death [1, 8]. Injuries to peripheral nerves lead to partial or complete loss of autonomic functions as well as motor and sensory functions. These dysfunctions that occur as a result of damage to peripheral nerves significantly reduce the quality of life in individuals. Therefore, it occupies an important place in rehabilitation medicine [1]. Unfortunately, treating the initial injury due to trauma in peripheral nerves is not enough to permanently heal the nerve. Secondary injury that occurs after trauma should be prevented. For this reason, effective therapeutic agents that can support surgical repair and physical rehabilitation, which are widely used in the treatment of peripheral nerve injury today, are sought to treat secondary injury [16, 19, 28]. In this context, in this study, the effectiveness of cold pressure atmospheric plasma, which has been shown to have healing and reparative effects in many tissues, on sciatic nerve injury in rats was investigated. When the results were evaluated histopathologically at the light microscopic level, it was observed that the general structure and axons in the sham group had normal morphologic structure. In all other groups, there were more degenerated axons, periaxonal swelling and decreased axonal density compared to the sham group. The borders of the axons in these groups could not be clearly distinguished and possible degenerated cell debris structures were frequently observed. The hot plate test is the most common procedure used to determine nociception in rats with peripheral nerve injuries [31]. When we looked at our hot plate test results, we observed a significant improvement in the hot plate test time in the group in which we applied 10 min cold pressure atmospheric plasma jet after our third plasma application (at the 3rd week after injury) and in the 10 min PAM group. The histopathological evaluations we performed are semi-quantitative. It is important for us to see the reversal of the primary injury process that occurs as a result of sciatic nerve injury. But we know that the main problem in peripheral nerve injuries is the secondary injury that develops due to the primary injury. In the process of secondary injury, oxidative stress and inflammation increase in the tissue. Due to increased oxidative and inflammatory damage, cell death pathways are activated and cells die [13].

When we evaluate all our results, we think that we should support our study with quantitative studies in order to evaluate the effectiveness of cold pressure atmospheric plasma on sciatic nerve injury more accurately. Oxidative stress, inflammation and cell death processes that occur during the secondary injury process in the tissue should also be evaluated. Lee et al. [23], examined the role of non-thermal plasma in sciatic nerve healing after making a complete incision in the sciatic nerve of rats. They applied non-thermal plasma to the treatment groups for 5 min three times a week for eight weeks. They evaluated functional recovery with the footprint test and observed that there were differences in the sciatic nerve index between the injury group and the group treated with non-thermal plasma only after the 4^{th} week. After eight weeks, they found that the treatment group could spread their toes much better and almost 60% of the function had returned. The researchers supported their results with detailed immunohistochemical analysis. In line with their results, they stated that non-thermal plasma treatment of sciatic nerve incisions increased motor function, accelerated myelination and axonal regeneration, and improved neuronal structure. The duration of the experiment and the frequency of plasma application were much longer than in our study. Plasma treatment applied only once a week for four weeks may have been insufficient to improve sciatic nerve damage. Another study has shown that non-thermal plasma may be an effective therapeutic agent in the treatment of sciatic nerve crush injury [22]. The researchers applied non-thermal plasma for 5 minutes three times a week for three weeks. After nine treatments (on day 21), they found that the rats in the treatment group could spread their toes much better than the rats in the damage group. They also examined the muscle and nerve tissues with detailed histological analysis. They performed immunofluorescent staining with macrophage marker CD68 protein and type I collagen antibodies to evaluate the effectiveness of plasma on the inflammation process that delays the healing process in muscle tissue. They found that type I collagen expression and CD68 expressing cells were decreased in the treatment group. When they examined the sciatic nerve tissue histologically, they found that the nine plasma treatments were insufficient to reduce the nerve tissue to normal thickness, but the density of nerve fibers was higher than in the damage group. To confirm the functional improvement in the nerve tissue, they performed immunofluorescent staining with specific antibodies that are markers for axon filaments and myelinated Schwann cells. Their analysis revealed that the myelin sheath actively healed and bridges connecting axon filaments were formed in the treatment group [22]. Again, unlike our study, the frequency and number of plasma applications were much higher in this study. In our study, it is seen that the low frequency of plasma application affected our results. Plasma applied once a week was

undoubtedly insufficient to heal the primary damage in the nerve tissue. Considering the other studies, plasma application at least three times a week would have allowed us to obtain more effective and clearer results. We also came to the conclusion that we should definitely evaluate the secondary injury process.

CONCLUSIONS

It is very important to reveal the effects of plasma application on oxidative damage and inflammation process occurring in the tissue in order to reliably evaluate the effectiveness of plasma. In our future studies on this subject, we plan to reveal the effectiveness of plasma with more comprehensive advanced kinetic and experimental analyzes by closing the deficiencies we have seen in this study.

Article information and declarations

Data availability statement

For ethical reasons, data cannot be shared.

Ethics statement

The study was carried out with the decision of the local ethics committee of Karabük University Animal experiments dated 27/12/2022 and numbered 2022/10. The rats used in the study were obtained from Karabük University Experimental Medicine Application and Research Center.

Author contributions

Idea: NY, YS, FB; Design: NY, YS, SS; Supervision: NY; Data collecting: NY, YS, SS, FB, OGD; Data prossesing: NY, YS, SS, FB, OGD; Analysis: NY, YS, SS, FB, OGD; Critical review: NY, YS, SS; Writing: NY, YS, SS, FB, OGD.

Funding

This study was supported by Karabuk University Scientific Research Projects Coordinatorship with project number KBÜBAP-23-DS-049.

Conflict of interest: None declared.

REFERENCES

1. An Y, Yan HX, Zhao JN, et al. Evaluation methods of a rat sciatic nerve crush injury model. J Integr Neurosci. 2022; 21(3): 91, doi: 10.31083/j.jin2103091, indexed in Pubmed: 35633172.
2. Attri P, Park JiH, Ali A, et al. How does plasma activated media treatment differ from direct cold plasma treatment? Anticancer Agents Med Chem. 2018; 18(6): 805–814, doi: 10.2174/1871520618666180406121734, indexed in Pubmed: 29623855.
3. Babaeva N, Kushner M. Reactive fluxes delivered by dielectric barrier discharge filaments to slightly wounded skin. J Phys D: Appl Phys. 2012; 46(2): 025401, doi: 10.1088/0022-3727/46/2/025401.
4. Boeckmann L, Bernhardt T, Schäfer M, et al. Plasma medicine: applications of cold atmospheric pressure plasma in dermatology. Oxid Med Cell Longev. 2019; 2019(2): 3873928–113, doi: 10.1155/2019/3873928, indexed in Pubmed: 31565150.
5. Canatan F. Atmosferik basınç plazma ile ilaç tedavisinin meme kanseri hücreleri üzerine etkilerinin karşılaştırılması: Lisansüstü Eğitim Enstitüsü.
6. Cha S, Park YS. Plasma in dentistry. Clin Plasma Med. 2014; 2(1): 4–10, doi: 10.1016/j.cpme.2014.04.002, indexed in Pubmed: 27030818.
7. Cheng YJ, Lin CK, Chen CY, et al. Plasma-activated medium as adjuvant therapy for lung cancer with malignant pleural effusion. Sci Rep. 2020; 10(1): 18154, doi: 10.1038/s41598-020-75214-2, indexed in Pubmed: 33097755.
8. Durak MA, Ozhan O, Tetik B, et al. Effects of apocynin on sciatic nerve injury in rabbits. Biotech Histochem. 2023; 98(3): 172–178, doi: 10.1080/10520295.2022.2146195, indexed in Pubmed: 36440649.
9. Ermolaeva SA, Varfolomeev AF, Chernukha MYu, et al. Bactericidal effects of non-thermal argon plasma in vitro, in biofilms and in the animal model of infected wounds. J Med Microbiol. 2011; 60(Pt 1): 75–83, doi: 10.1099/jmm.0.020263-0, indexed in Pubmed: 20829396.
10. Gan Lu, Duan J, Zhang S, et al. Cold atmospheric plasma ameliorates imiquimod-induced psoriasiform dermatitis in mice by mediating antiproliferative effects. Free Radic Res. 2019; 53(3): 269–280, doi: 10.1080/10715762.2018.1564920, indexed in Pubmed: 30663913.
11. He R, Li Q, Shen W, et al. The efficacy and safety of cold atmospheric plasma as a novel therapy for diabetic wound in vitro and in vivo. Int Wound J. 2020; 17(3): 851–863, doi: 10.1111/iwj.13341, indexed in Pubmed: 32168435.
12. Heinlin J, Morfill G, Landthaler M, et al. Plasma medicine: possible applications in dermatology. J Dtsch Dermatol Ges. 2010; 8(12): 968–977, doi: 10.1111/j.1610-0387.2010.07495.x, indexed in Pubmed: 20718902.
13. Henry JL. Mechanisms of peripheral nerve injury – what to treat, when to treat. IntechOpen, London 2014.
14. Hoffmann C, Berganza C, Zhang J. Cold Atmospheric Plasma: methods of production and application in dentistry and oncology. Med Gas Res. 2013; 3(1): 21, doi: 10.1186/2045-9912-3-21, indexed in Pubmed: 24083477.

15. Isbary G, Shimizu T, Li YF, et al. Cold atmospheric plasma devices for medical issues. Expert Rev Med Devices. 2013; 10(3): 367–377, doi: 10.1586/erd.13.4, indexed in Pubmed: 23668708.
16. Iwahashi T, Suzuki K, Tanaka H, et al. Neurotropin® accelerates peripheral nerve regeneration in a rat sciatic nerve crush injury model. J Orthop Sci. 2024; 29(2): 653–659, doi: 10.1016/j.jos.2023.02.002, indexed in Pubmed: 36858838.
17. Keidar M, Walk R, Shashurin A, et al. Cold plasma selectivity and the possibility of a paradigm shift in cancer therapy. Br J Cancer. 2011; 105(9): 1295–1301, doi: 10.1038/bjc.2011.386, indexed in Pubmed: 21979421.
18. Kim YJ, Lim DJ, Lee MiY, et al. Prospective, comparative clinical pilot study of cold atmospheric plasma device in the treatment of atopic dermatitis. Sci Rep. 2021; 11(1): 14461, doi: 10.1038/s41598-021-93941-y, indexed in Pubmed: 34262113.
19. Korkmaz MF, Parlakpinar H, Erdem MN, et al. The therapeutic efficacy of dexpanthenol on sciatic nerve injury in a rat model. Br J Neurosurg. 2020; 34(4): 397–401, doi: 10.1080/02688697.2020.1749984, indexed in Pubmed: 32297525.
20. Laroussi M. Nonthermal decontamination of biological media by atmospheric-pressure plasmas: review, analysis, and prospects. IEEE Trans Plasma Sci. 2002; 30(4): 1409–1415, doi: 10.1109/tps.2002.804220.
21. Laroussi M, Mohades S, Barekzi N. Killing adherent and nonadherent cancer cells with the plasma pencil. Biointerphases. 2015; 10(2), doi: 10.1116/1.4905666.
22. Lee HG, Choi JH, Jang YS, et al. Non-thermal plasma accelerates the healing process of peripheral nerve crush injury in rats. Int J Med Sci. 2020; 17(8): 1112–1120, doi: 10.7150/ijms.44041, indexed in Pubmed: 32410841.
23. Lee ST, Jang YS, Kim UK, et al. Non-thermal plasma application enhances the recovery of transected sciatic nerves in rats. Exp Biol Med (Maywood). 2021; 246(11): 1287–1296, doi: 10.1177/1535370221996655, indexed in Pubmed: 33653158.
24. Li M, Gao J, Wang L, et al. Basic research and clinical exploration of cold atmospheric plasma for skin wounds. Bioeng Transl Med. 2023; 8(5): e10550, doi: 10.1002/btm2.10550, indexed in Pubmed: 37693064.
25. Liu J, Yang C, Cheng C, et al. In vitro antimicrobial effect and mechanism of action of plasma-activated liquid on planktonic . Bioengineered. 2021; 12(1): 4605–4619, doi: 10.1080/21655979.2021.1955548, indexed in Pubmed: 34320914.
26. Maho T, Binois R, Brulé-Morabito F, et al. Anti-Bacterial action of plasma multi-jets in the context of chronic wound healing. Applied Sciences. 2021; 11(20): 9598, doi: 10.3390/app11209598.
27. Moisan M, Barbeau J, Crevier MC, et al. Plasma sterilization. Methods and mechanisms. Pure Appl Chem. 2009; 74(3): 349–358, doi: 10.1351/pac200274030349.
28. Ogut E, Yildirim FB, Sarikcioglu L, et al. Neuroprotective effects of ozone therapy after sciatic nerve cut injury. Kurume Med J. 2020; 65(4): 137–144, doi: 10.2739/kurumemedj.MS654002, indexed in Pubmed: 31391380.

29. Ozdemir E, Gursoy S, Bagcivan I. The effects of serotonin/norepinephrine reuptake inhibitors and serotonin receptor agonist on morphine analgesia and tolerance in rats. J Physiol Sci. 2012; 62(4): 317–323, doi: 10.1007/s12576-012-0207-x, indexed in Pubmed: 22544464.

30. Özdemir A. Soğuk Atmosferik Plazma ve Kanser. Researcher. 2021; 1(2): 6–18.

31. Ozhan O, Izci SF, Huz M, et al. Therapeutic effects of cinnamon bark oil on sciatic nerve injury in rats. Eur Rev Med Pharmacol Sci. 2023; 27(12): 5841–5853, doi: 10.26355/eurrev_202306_32823, indexed in Pubmed: 37401321.

32. Oztan MO, Ercan UK, Aksoy Gokmen A, et al. Irrigation of peritoneal cavity with cold atmospheric plasma treated solution effectively reduces microbial load in rat acute peritonitis model. Sci Rep. 2022; 12(1): 3646, doi: 10.1038/s41598-022-07598-2, indexed in Pubmed: 35256655.

33. Rasouli M, Mehdian H, Hajisharifi K, et al. Plasma activated medium induces apoptosis in chemotherapy-resistant ovarian cancer cells: high selectivity and synergy with carboplatin. Plasma Processes and Polymers. 2021; 18(9), doi: 10.1002/ppap.202100074.

34. Rodríguez F, Valero-Cabré A, Navarro X. Regeneration and functional recovery following peripheral nerve injury. Drug Discov Today: Dis Models. 2004; 1(2): 177–185, doi: 10.1016/j.ddmod.2004.09.008.

35. Salehi M, Naseri-Nosar M, Ebrahimi-Barough S, et al. Regeneration of sciatic nerve crush injury by a hydroxyapatite nanoparticle-containing collagen type I hydrogel. J Physiol Sci. 2018; 68(5): 579–587, doi: 10.1007/s12576-017-0564-6, indexed in Pubmed: 28879494.

36. Scholtz V, Pazlarova J, Souskova H, et al. Nonthermal plasma — a tool for decontamination and disinfection. Biotechnol Adv. 2015; 33(6 Pt 2): 1108–1119, doi: 10.1016/j.biotechadv.2015.01.002, indexed in Pubmed: 25595663.

37. Tendero C, Tixier C, Tristant P, et al. Atmospheric pressure plasmas: A review. Spectrochim Acta Part B At Spectrosc. 2006; 61(1): 2–30, doi: 10.1016/j.sab.2005.10.003.

38. Tušek L, Nitschke M, Werner C, et al. Surface characterisation of NH3 plasma treated polyamide 6 foils. Colloids Surf A: Physicochem Eng Asp. 2001; 195(1-3): 81–95, doi: 10.1016/s0927-7757(01)00831-7.

39. Vecchio D, Dai T, Huang L, et al. Antimicrobial photodynamic therapy with RLP068 kills methicillin-resistant Staphylococcus aureus and improves wound healing in a mouse model of infected skin abrasion PDT with RLP068/Cl in infected mouse skin abrasion. J Biophotonics. 2013; 6(9): 733–742, doi: 10.1002/jbio.201200121, indexed in Pubmed: 22987338.

40. Xiao H, Wei C, Liu H, et al. Lentinan alleviates sciatic nerve injury by promoting autophagy to remove myelin fragments. Phytother Res. 2023; 37(9): 4042–4058, doi: 10.1002/ptr.7862, indexed in Pubmed: 37165703.

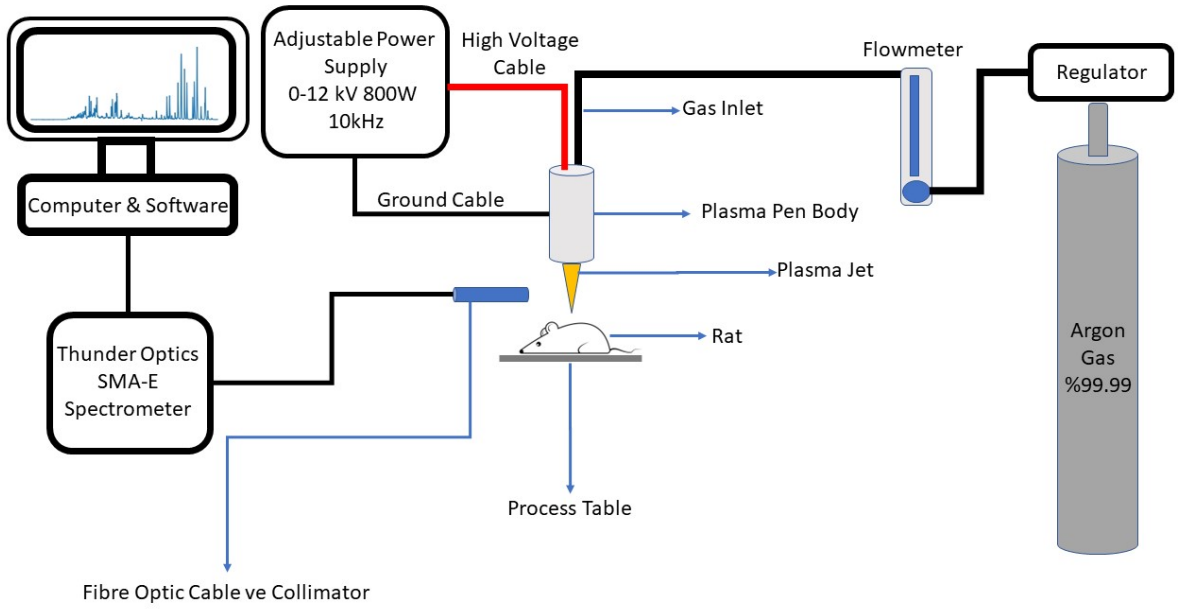

Figure 1. Schematic representation of the plasma system

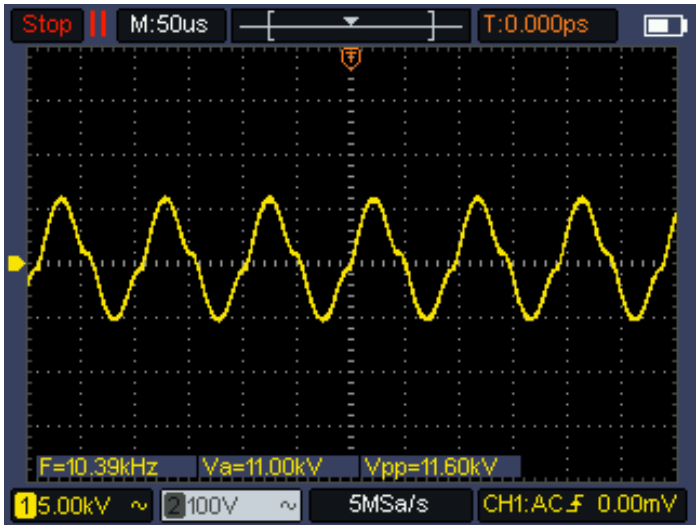

Figure 2. Oscilloscope image

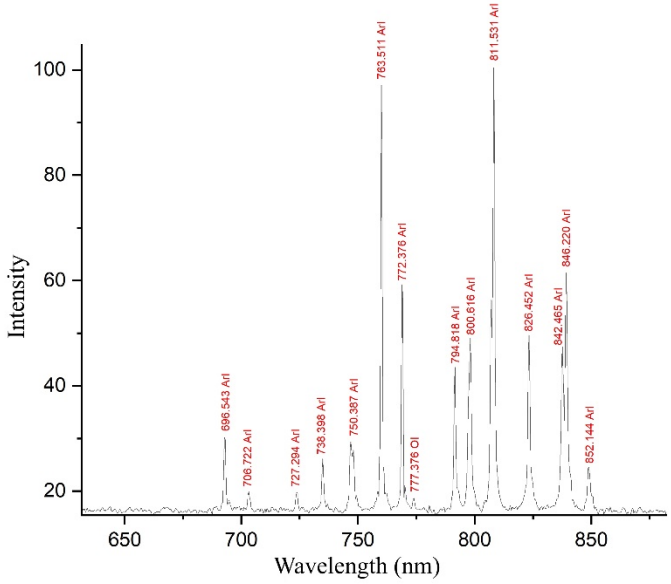

Figure 3. Optical emission spectrum

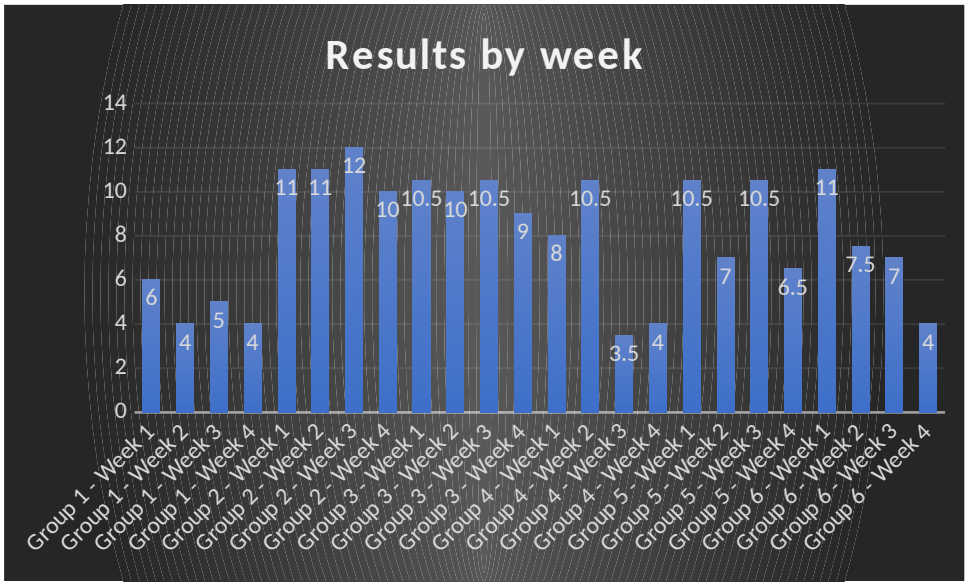

Figure 4. Hot plate test results according to weeks

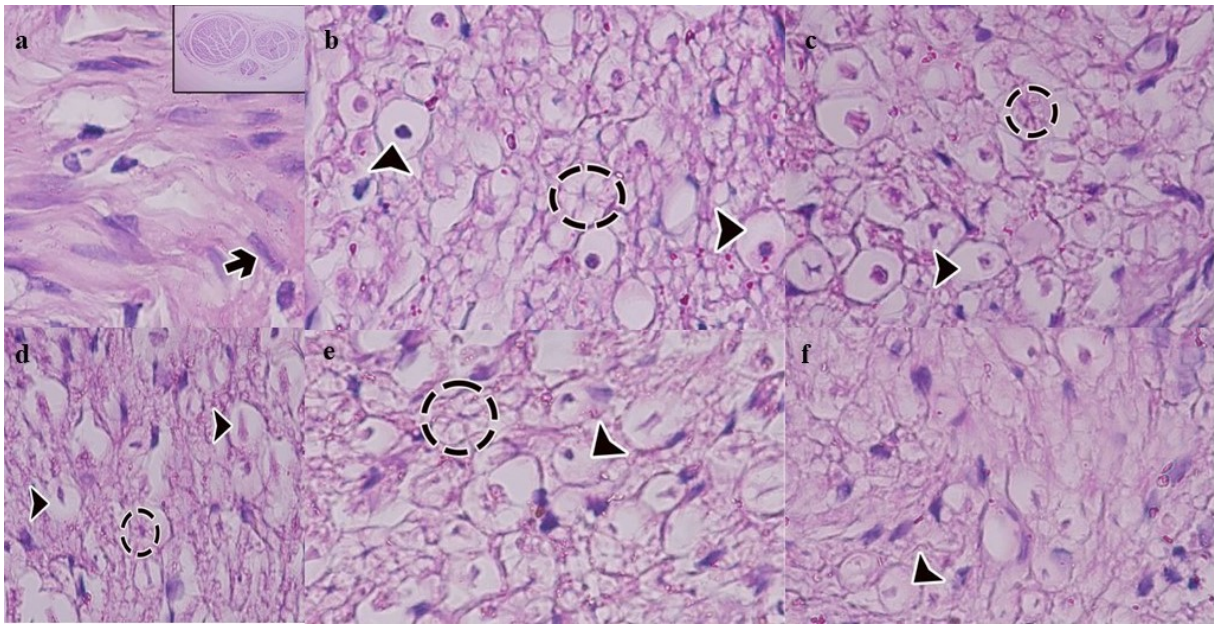

Figure 5. Light microscopic image of rat sciatic nerve tissue (a: Group 1, b: Group: 2, c: Group 3, d: Group 4, e: Group 5, f: Group 6, Arrow: Healthy axon; Head of arrow: Periaxonal swelling; Circle mark: axonal degeneration).

Table 1. Descriptive statistics and group comparisons according to total hot plate test averages

Groups	Median (Minimum-Maximum)
Group 1 (Sham group)	4.375 (3.5–5) [a]
Group 2 (Sciatic nerve injury [SNI] group)	11.10 (8.50–14.30) [a, b]
Group 3 (SNI + Atmospheric pressure cold plasma jet 5 min)	9.375 (7–11.75) [a]
Group 4 (SNI + Atmospheric pressure cold plasma jet 10 min)	7.5 (2.5–11) [a, b]
Group 5 (SNI + Atmospheric pressure cold plasma activated (PAM) 5 min)	8.75 (5.25–12.5) [a, b]
Group 6 (SNI + Atmospheric pressure cold plasma activated (PAM) 10 min)	8.375 (5–10.75) [a, b]

[a] $p < 0.05$ Group 1 vs 2, 3, 4, 5, 6

[b] $p < 0.05$ Group 2 vs 4, 5, 6

Table 2. Hot plate test results according to weeks and comparison between groups

Groups	Weeks	Median (Minimum-Maximum)
Group 1 (Sham group)	Week 1	6 (2–7)[a]
	Week 2	4 (2–6)[c]
	Week 3	5 (3–5)[e]
	Week 4	4 (2–5)[i]
Group 2 (Sciatic nerve injury [SNI] group)	Week 1	11 (10–15)[a, b]
	Week 2	11 (10–15)[c, d]
	Week 3	12 (9–15)[e, f]
	Week 4	10 (4–15)[i, j]
Group 3 (SNI + Atmospheric pressure cold plasma jet 5 min)	Week 1	10.5 (6–15)[a]
	Week 2	10 (5–11)[c]
	Week 3	10.5 (2–12)[e, g]
	Week 4	9 (3–15)[i]
Group 4 (SNI + Atmospheric pressure cold plasma jet 10 min)	Week 1	8 (4–15)[b]
	Week 2	10.5 (1–15)[c]
	Week 3	3.5 (2–15)[f, g, h]
	Week 4	4 (2–15) [j]
Group 5 (SNI + Atmospheric pressure cold plasma activated (PAM) 5 min)	Week 1	10.5 (6–13)[a]
	Week 2	7 (2–15)[c, d]
	Week 3	10.5 (3–15)[e, h]
	Week 4	6.5 (2–11)
Group 6 (SNI + Atmospheric pressure cold plasma activated	Week 1	11 (5–12)[a]
	Week 2	7.5 (4–15)[c, d]

(PAM) 10 min)	Week 3	7 (3–15)
	Week 4	4 (2–13) [j]

[a] p < 0.05 Group 1 vs 2, 3, 5, 6; [b]p < 0.05 Group 2 vs 4; [c]p < 0.05 Group 1 vs 2, 3, 4, 5, 6; [d]p < 0.05 Group 2 vs 5, 6; [e]p < 0.05 Group 1 vs 2, 3, 5; [f]p < 0.05 Group 2 vs 4; [g]p < 0.05 Group 3 vs 4; [h]p < 0.05 Group 4 vs 5; [i]p < 0.05 Grup 1 vs 2, 3, 4; [j]p < 0.05 Group 2 vs 4, 6

Table 3. Histopathological analysis results

Histopathologic changes	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**p**
Periaxonal swelling	1 (0,75–1)[a]	3 (2–3)[b]	2.5 (2–3)[b]	2 (2–3)[b]	2 (1.75–3)[b]	3 (2–3)[b]	0.001
Axonal degeneration	0.5 (0–1)[a]	2 (1.75–3)[b]	2 (2–3)[b]	3 (1.75–3)[b]	2.5 (2–3)[b]	2.5 (2–3)[b]	0.001
Axonal density	1 (0–1)[a]	3 (2–3)[b]	2.5 (2–3)[b]	2 (2–3)[b]	2.5 (2–3)[b]	3 (2–3)[b]	0.001