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Development and Characterization of a Novel Microwave Plasma Source for Enhanced Healing in Wound Treatment

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Abstract: Our study explores the potential of a novel microwave plasma source for enhancing wound healing in BALB-C mouse models. Chronic wounds, particularly in diabetic individuals, present significant challenges due to impaired regenerative capacity. Cold Atmospheric Plasma (CAP) has emerged as a promising approach, offering diverse therapeutic benefits. However, its specific efficacy in the context of diabetic wounds remains underexplored. We developed and characterized a microwave plasma source optimized for wound treatment, inducing acute wounds and treating them with CAP in a controlled experimental setup. The treated group exhibited accelerated wound closure compared to controls, suggesting CAP's potential to enhance the healing process. Our findings underscore CAP's multifaceted impact on the wound healing cascade, highlighting its ability to promote angiogenesis, modulate inflammatory responses, and exhibit antimicrobial properties. These results position CAP as a promising intervention in acute wound management, paving the way for further exploration of its therapeutic potential in clinical settings.

Keywords: cold atmospheric plasma; microwave plasma; plasma device; wound healing; tissue regeneration; microwave plasma; argon plasma; wound regeneration; wound care

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1. Introduction

Managing acute wounds has long posed a significant clinical challenge [1–3]. The regenerative capacity of tissues in acute wound conditions has been extensively researched [4,5]. Acute wounds are often characterized by inflammation, tissue regeneration, and a risk of infections [6–8]. Cold Atmospheric Plasma (CAP), a unique state of matter generating reactive oxygen and nitrogen species, has recently emerged as a promising approach to addressing this challenge [9,10]. CAP's diverse chemical composition and non-equilibrium nature offer a novel perspective in wound care. Operating at room temperature, Cold Atmospheric Plasma (CAP) is highly suitable for clinical use, producing a mix of reactive species that interact effectively with tissues [11–13]. These reactive species are capable of modulating cellular signaling pathways, reducing inflammation, enhancing angiogenesis, promoting collagen synthesis, and stimulating tissue regeneration—key elements in the wound healing process [14,15].

As CAP technology is relatively new in the medical field, it has garnered significant interest and spurred extensive research. Its distinctive properties and potential to overcome challenges associated with acute wounds have led to lively discussions in the scientific community. This research aims to deepen our understanding of CAP's mechanisms and therapeutic advantages in wound healing. CAP's potential to revolutionize wound care is promising, and this study aims to highlight its innovative potential as a treatment for wounds.

Acute wounds pose a significant healthcare challenge due to their complex healing requirements and high risk of complications, which substantially affect patient morbidity and healthcare expenses [16,17]. The pressing need for effective therapeutic interventions is underscored by ongoing scientific debates on the best strategies to enhance wound healing in acute conditions [18]. Despite various proposed and tested treatment approaches, a notable lack of consensus remains on the most effective way to manage acute wounds. This diversity stems from the complexity of underlying biological processes, patient heterogeneity, and variations in wound etiology. Additionally, within the context of acute wound regeneration, the specific impact of CAP, particularly when employed in an argon environment, has not been comprehensively studied [1,19,20]. CAP in argon is intriguing due to the unique interactions of plasma with biological objects in the presence of different gases, which may affect mechanisms of healing. Moreover, investigating CAP's effectiveness and mechanisms in the context of acute wounds, particularly using an argon setting, is expected to yield valuable insights into this innovative treatment approach.

Related studies on the application of CAP devices in BALB-C mouse models, as documented in References [21–24], explored CAP's therapeutic effects on wound healing and tissue regeneration. Notably, these studies used various devices and feeding gases. None of the devices in these referenced studies employed microwave technology. Instead, they used direct or alternating current with high-voltage technologies. This set of technologies highlights the wide range of approaches in CAP research for the healing process, providing important insights into the applications of various plasma technologies in addressing tissue complications.

In the field of CAP applications using BALB-C mouse models, the studies referenced as [21,22,24] are particularly significant, providing detailed accounts of the chronological progression of wound closure that align with the primary focus of our experiment. These studies thoroughly explored the dynamic process of tissue regeneration, offering a comprehensive understanding of the macroscopic changes that occur during wound healing over time. In contrast, the study cited as [23] focused on evaluating oxidative stress markers and specific proinflammatory cytokines. Despite its different primary focus, [23], along with [21,22,24], collectively contributed to uncovering the complex mechanisms involved in tissue regeneration. Each study enhances our understanding of the processes at play in healing wounds. Referenced papers [21–24] provided fundamentals for further studies of assisted wound healing by CAPs. Some of them focused on temperature effects [23], others—on the antibacterial and sterilizing effects of plasmas [22], or variations in effects by the health status of the animal models [23]. Leaving aside the differences in studies, what these articles have in common is the proof of the positive effect of plasma treatment on wound healing. These investigations' nuanced perspectives contribute to a more comprehensive understanding of the mechanisms underlying positive outcomes, offering potential explanations for CAP and related therapies' efficacy in acute wound regeneration.

Based on the latest breakthroughs in regenerative medicine and plasma-based therapies, particularly notable studies such as [25,26], this investigation represents a pivotal advancement in understanding how CAP can be utilized for treating acute wounds. Regenerative medicine, an area of rapid development, has introduced several novel therapies, with CAP capturing attention due to its distinct characteristics influencing regenerative processes. CAP's ability to generate reactive species that engage with biological tissues is particularly intriguing, presenting a promising avenue for transforming the management of acute wounds.

The development of our microwave Cold Atmospheric Plasma (MCAP) source is crucial because it offers a novel approach to wound healing that has the potential to address several key challenges. First, our plasma source operates at ambient temperatures, making it suitable for clinical applications without the risk of thermal damage to tissues. Additionally, it generates a mixture of reactive species that interact with biological tissues, modulating cellular signaling and promoting processes critical for wound healing, such as inflammation reduction, angiogenesis, collagen synthesis, and tissue regeneration. This unique capability of our plasma source opens up new possibilities for improving the outcomes of wound care, particularly in the context of acute wounds where rapid and effective healing is essential. Moreover, by advancing the understanding of plasma-based therapies, our development contributes to the broader landscape of regenerative medicine, offering potential solutions for a range of biomedical applications beyond wound healing. Last but not least, we should mark that our plasma source utilizes innovative design and materials, including being entirely manufactured from PLA (polylactic acid) material using a fuse depiction modeling (FDM) 3D printer. This not only reduces production costs but also allows for customization and optimization of the design for more efficient generation of microwave discharges at low microwave power. These factors contribute to achieving low temperatures that are optimal for wound treatment without compromising treatment effectiveness.

2. Materials and Methods

The schematic of the experimental setup is presented in Figure 1a. The CAP jet was generated through a plasma exciter. Microwave power was sourced by Sairem (Décines-Charpieu, France), a GMS 200 W microwave generator with an operational frequency of 2.45 GHz. Initial studies were undertaken with a microwave power between 5 and 30 W. Reflected power from the device did not exceed 1 W. The discharger, illustrated in Figure 1b,c, featured a metal antenna positioned at its axis. Argon was introduced by two inputs on the bottom of the discharge chamber. A plasma torch end was used for treatment. Depending on argon flow speed, the use of microwave power produced torches with lengths between 1 and 10 mm. The jet thickness was below 3 mm, while the discharge gap's diameter was 5 mm.

The temperature of the treated area was monitored during in vivo treatments using a Testo (Titisee-Neustadt, Germany) 865 infrared camera, as investigated in [27,28]. Temperature measurements were taken by reflecting radiation from a mica plate. A VÖGTLIN (Unna, Germany) RED-Y compact® mass flow controller was used to control gas flow speed between 2 and 12.5 L/min.

The developed discharge exciter was based on devices already registered as utility models (registration numbers 4601 U1 and 4602 U1) with the Patent Office of the Republic of Bulgaria. Previous results on wound healing in rat models using the utility model with registration number 4602 U1 have been presented in [28]. The new device was based on utility model 4602 U1 with an enhanced gas flow distribution, exchangeable gas discharge chamber, and varied antenna positioning. These registered designs provided a robust foundation for our current research, ensuring the reliability and efficacy of the exciter for enhanced wound treatment applications. Similar to the utility model on which the device was based, an FDM printer (BAMBULAB X1C, Shenzhen, China) was used, and all elements of the device were printed from PLA material. The low softening point of the material (around 60 °C) allowed for self-regulation of the device's internal temperature.

The length of the discharge was measured using a Canon (Tokyo, Japan) EOS Mark II camera equipped with a Carl Zeiss (Oberkochen, Germany) Macro-Planar 2/50 ZE lens. The calculated plasma torch length was averaged from more than six images taken under each set of discharge conditions to ensure accuracy. Emission spectra were collected using an STS-UV miniature spectrometer from Ocean Optics, providing detailed spectral data for further analysis of the plasma characteristics.

Ten male BALB-C mice weighing between 65 and 80 g each were used in the experiments. The source of the laboratory animals was the Vivarium of Medical University–Sofia. The mice were individually housed in cages under standard laboratory conditions: 12-h light–dark cycle; 24 °C room temperature; 55% humidity; and no restriction of access to standard laboratory chow and water during the study.

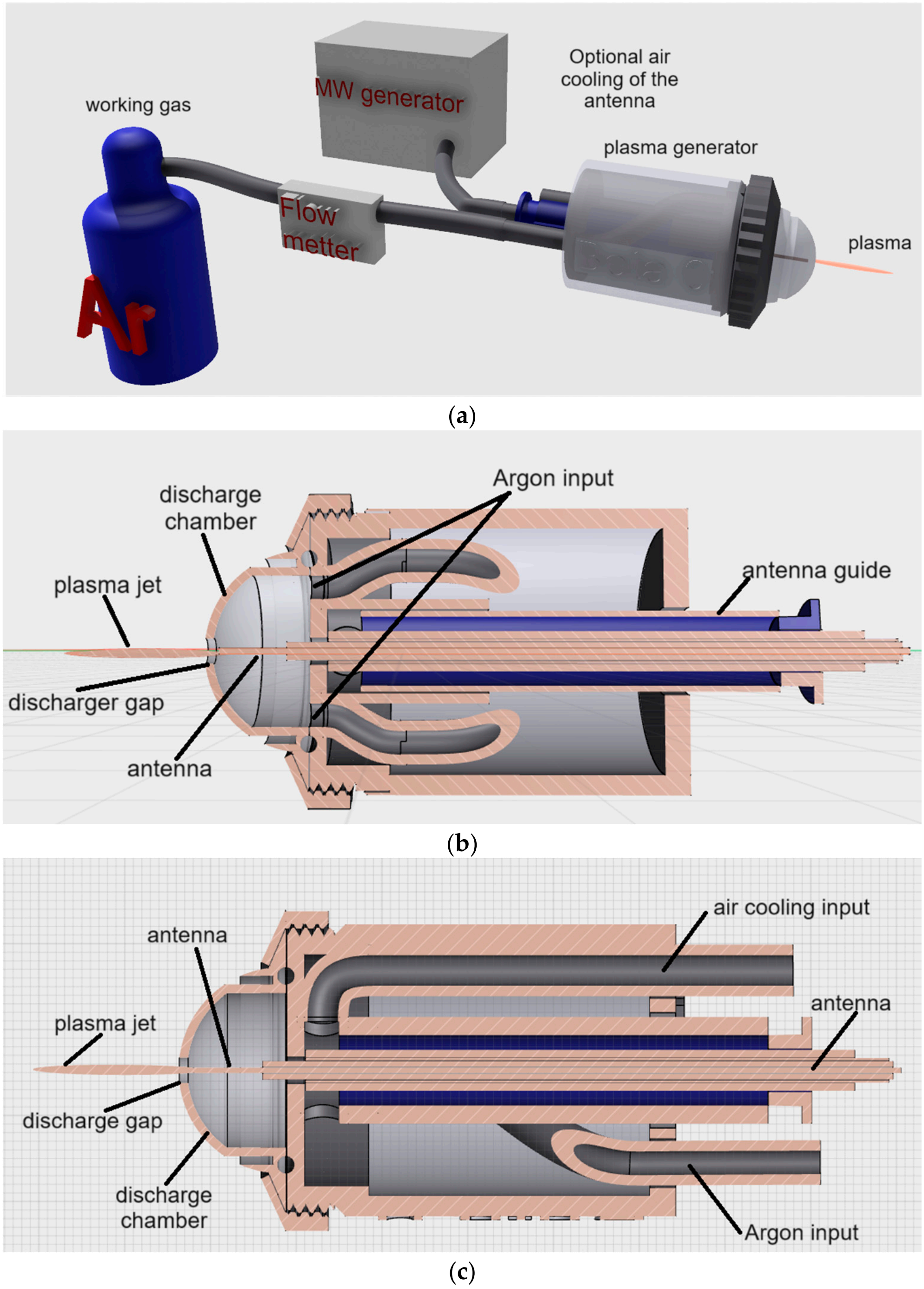


Figure 1. Scheme of microwave plasma torch device (**a**) and axial cuts of the exciter (**b**,**c**).

After a one-week habituation, we divided mice into 2 groups, with 5 mice in each group. One group served as the treated group (MT) and the other as the control group (MC). Mice in the treated group received treatment 2 h after the injury, while those in the control group did not receive any treatment. Circular-shaped wounds on the epidermal layer, each with an area of 3.5 cm^2, were induced while the animals were anesthetized.

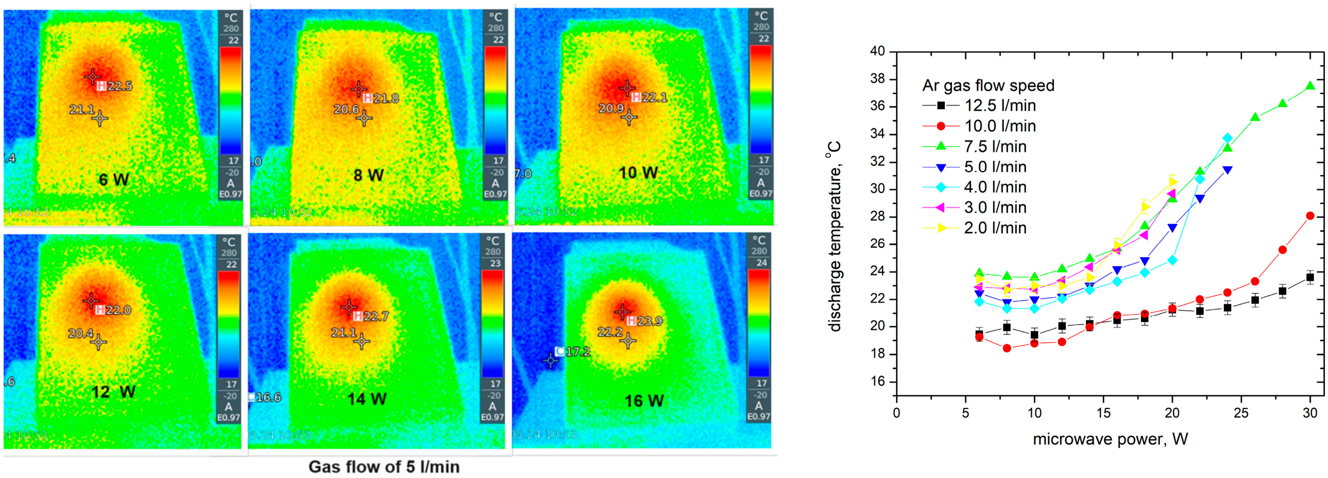
The treatment protocol involved exposing the wound site to argon microwave Cold Atmospheric Plasma (MCAP) once, with the session lasting 15 s. The treatment conditions were set at an applied power of 10 W and a gas flow of 5 L/min. The parameters were chosen based on experimental results concerning the plasma torch length and temperature, ensuring precise and controlled CAP delivery to prevent potential side effects. Reduction in wound area into the time was measured once per day for the first 3 days in a row, then each second day until the 25th-day post-treatment.

ImageJ 1.54h software was used to define the area of the wounds from several images of injury each time. Images were collected with a Canon EOS Mark II camera. To ensure precision, a calibration ruler which is visible in the images was utilized. A reference scale was used to convert pixels into metric units. More than 5 images per certain study of each mouse were manually processed to define wound area reduction. A special 3D-printed restrainer with sizes suitable for the studied mice was prepared and used. The methodology which was used was the same as the one already presented in [28].

The research involving BALB-C mice in this study was undertaken according to the guidelines and regulations established by the Bulgarian Food Safety Agency. Permission number 182 ensured compliance with all ethical and legal considerations by the researchers. Qualification of the researchers for humane treatment of animals in experiments, provided by the National Control and Education Institution, guarantees following the triple-R (Replacement, Reduction, and Refinement) principle.

3. Results and Discussion

The temperature of the MCAP-treated area was closely monitored using thermography on a mica plate, with results shown in Figure 2. The graphically presented results for the gas temperature of the plasma represent averaged values from five separate measurements with exposure to the mica plate over 30 s. Our study revealed that, under the conditions tested, the MCAP temperature was below 38 °C. This optimal temperature was consistently maintained when the applied power was at or below 25 W, and the gas flow was at least 2 L/min. The thermographic analysis of the mica plate visually confirmed this regulation, ensuring that MCAP operated within a biocompatible temperature range, thus minimizing the risk of thermal damage to the skin. The results highlighted the precise control of experimental parameters, providing valuable insight into the safe and effective application of CAP in various biological contexts. Additionally, the figure demonstrates that increasing microwave power resulted in a rise in plasma torch temperature across all tested gas flow rates.



(a) (b)

Figure 2. Thermographic images of the treated area's temperature at different microwave power levels and 5 L/min gas flow (**a**) and graphical representation of plasma's temperature for various applied powers and Ar flow (**b**).

Careful control of the MCAP temperature is crucial in in-vivo experiments. Ensuring an optimal temperature is important to prevent a heat effect. Figure 3 graphically demonstrates that the plasma temperature remained within the range of mouse body temperature throughout the experimental duration.

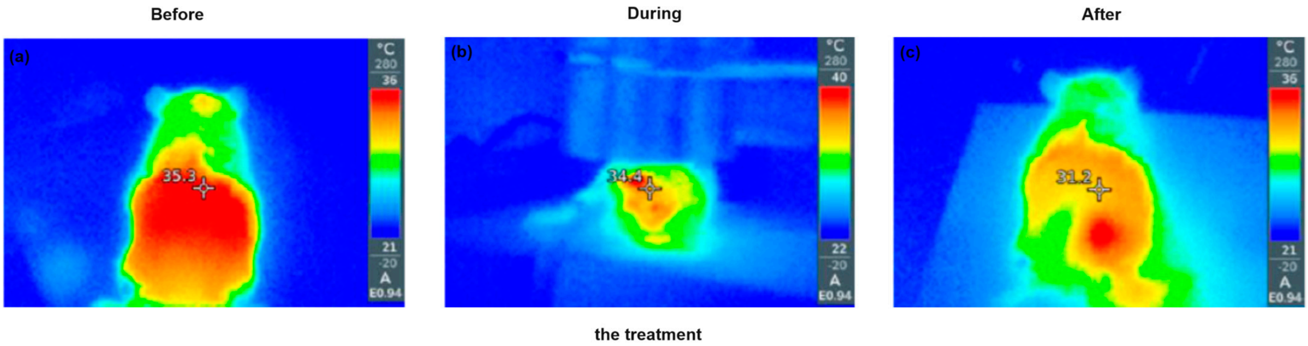


Figure 3. Infrared thermography of the mouse's body before (**a**), during (**b**), and after (**c**) the treatment with Ar MCAP applied power of 10 and 5 L/min Ar flow.

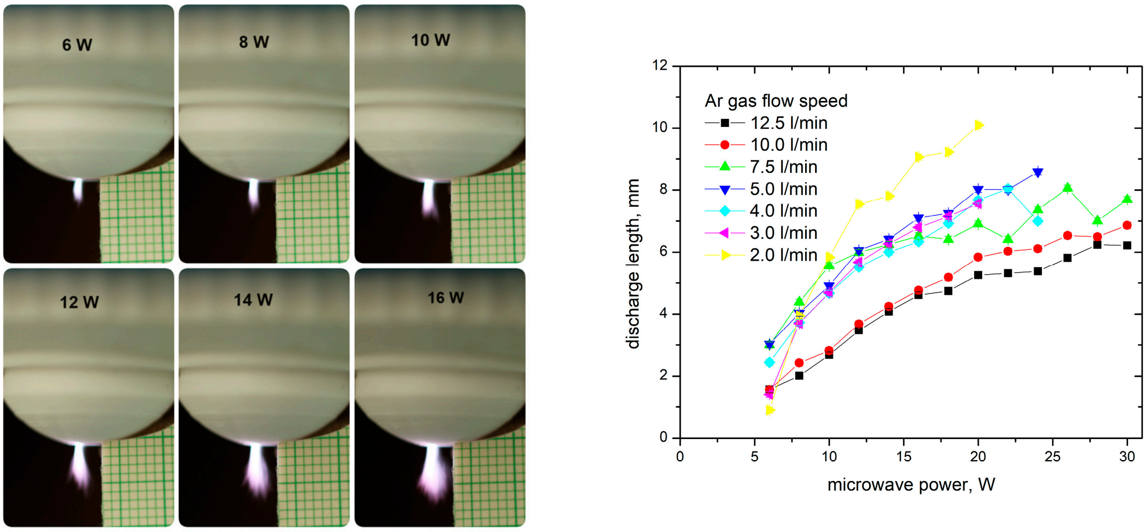
Detailed analysis of the thermographic images provided some conclusions about the relationship between MCAP temperature and the experimental parameters. Increasing the applied microwave power led to a noticeable rise in temperature. Reduction in gas flow resulted in an increase in temperature, which was the opposite of the presented results in [28]. A conclusion that variation in microwave power is the dominant temperature affect parameter in comparison with gas flow can be drawn.

In our analysis, we observed gas heating within the jet, which may be due to fast gas heating mechanisms. This phenomenon is especially important in environments with an admixture of air consisting of oxygen and nitrogen, as in our case. According to study [29], fast gas heating occurs through mechanisms such as electron impact dissociation. In this process, high-energy electrons collide with nitrogen molecules, causing them to dissociate and release energy, contributing to the rapid heating of the gas. Additionally, the quenching of excited nitrogen molecules by oxygen molecules is another critical mechanism. As discussed by [30], this quenching process involves the transfer of energy from excited nitrogen to oxygen, resulting in the release of energy and an increase in gas temperature.

Our experimental observations were not inconsistent with these findings, although the study was not specifically aimed at determining the reactions leading to the formation of thermal radiation from plasma. It was indicative that the presence of air in the inert gas stream significantly enhanced the gas heating effect. This is likely due to the efficient energy transfer during the quenching process, leading to the release of significant thermal energy. The implications of these mechanisms are crucial for accurately modeling the thermal dynamics in our jet system and should be taken into account in subsequent discharge studies, including modeling ones. Understanding the contribution of fast gas heating should allow for more precise predictions of temperature distributions and energy transfer processes.

Furthermore, these mechanisms highlighted the importance of considering the specific gas composition and discharge conditions in our experiments. This was not the subject of the current work, but subsequent research on discharge in the atmosphere inevitably needs to consider it. The insights provided by [29,30] emphasized the need to account for fast heating effects when analyzing gas dynamics in similar setups. By incorporating these considerations into our future modeling studies, we can achieve a more comprehensive understanding of the thermal behavior in plasma and jet environments. Therefore, future studies should continue to explore these mechanisms in greater detail to refine our knowledge of fast gas heating and its impact on gas heating dynamics.

The observed increase in temperature with reduced gas flow can be explained by the behavior of the plasma torch. As shown in Figure 4a, the discharge became stratified with an increase in applied microwave power, while the length of the discharge did not significantly extend. Figure 4b further illustrates that the plasma column length varied with gas discharge conditions, showing that higher microwave power leads to a longer plasma column. This stratification and elongation behavior under different power and flow conditions highlighted the intricate dynamics of the plasma torch, contributing to the overall thermal characteristics observed during CAP treatment.



(**a**) (**b**)

Figure 4. Experimental results about the plasma torch length at different microwave power and 5 L/min gas flow (**a**) and for various applied power and Ar flow (**b**).

Comparing the results in Figures 2b and 4b, similarities can be observed in the behavior of the curves for temperature and plasma jet length. For both studied parameters, an increase was noted with the rise in microwave power, but the curves tend to group at lower gas flow rates (below 10 L/min) and higher ones (above 10 L/min). It was clearly visible at applied low microwave power. In the figures, the black and red curves, representing gas flow above 10 L/min, exhibited a slower increase in plasma length and temperature with a rise in the applied microwave power compared to the discharges with lower gas flow rates. This grouping illustrated that lower gas flows result in more gradual changes in the plasma characteristics, whereas higher gas flows lead to more pronounced increases in plasma length and temperature. These observations suggested that the gas flow rate significantly influences the behavior of the plasma torch, with higher flow rates contributing to a more rapid extension and heating of the plasma column. This stratification of results highlights the intricate interplay between gas flow and microwave power, providing a deeper understanding of the plasma dynamics under varying discharge conditions.

A detailed understanding of how microwave power and gas flow affect MCAP temperature expands our knowledge of devices and underpins the precise selection of gas discharge conditions for various device application goals.

Based on the observed results, we selected a targeted approach with an applied microwave power of 10 Watts, a gas flow of 5 L/min, and a treatment duration not exceeding 15 s per session. These parameters were carefully chosen to balance therapeutic efficacy with the safety of the treated tissues. This approach of pre-estimating the effect of input parameters on discharge characteristics was intended to maximize the benefits of CAP while minimizing the potential risks that would occur with longer exposure.

Using optical emission spectroscopy (OES) with low spectral resolution capability of the instrument allowed for rapid screening of the presented radicals in the plasma column and its vicinity. The spectral resolution of over 2.2 nm did not enable quantitative analysis and determination of electron concentration and temperature, for example, but served as a good indicator of the plasma's potential to interact with wounds and potentially facilitate a healing process due to the possible presence of active oxygen and nitrogen radicals. We identified the chemical composition in the argon plasma torch. Detailed qualitative analysis of OESs in the range from 300 to 850 nm was presented for the plasma torch without contact and in contact with the skin. Lines have been identified in the spectrum that belonged to excited OH radicals, nitrogen, and atomic oxygen, and one with those of argon. All these radicals that were produced by the plasma and its interaction with the surrounding gas were transferred and implemented in the skin. Their ability to convert into other reactive oxygen species (ROS) and reactive nitrogen species (RNS) is also known.

In addition, OH and N_2 emissions were observed. Strong emission lines of argon at high wavelengths were observed in both studied spectra. The graphs in Figure 5 showed intense emissions of atomic oxygen that should be due to moisture in the atmosphere. The difference between the OES of the plasma torch and the OES of the skin contact torch was in the intensity of the lines, with no significant difference in the current lines between the OESs.

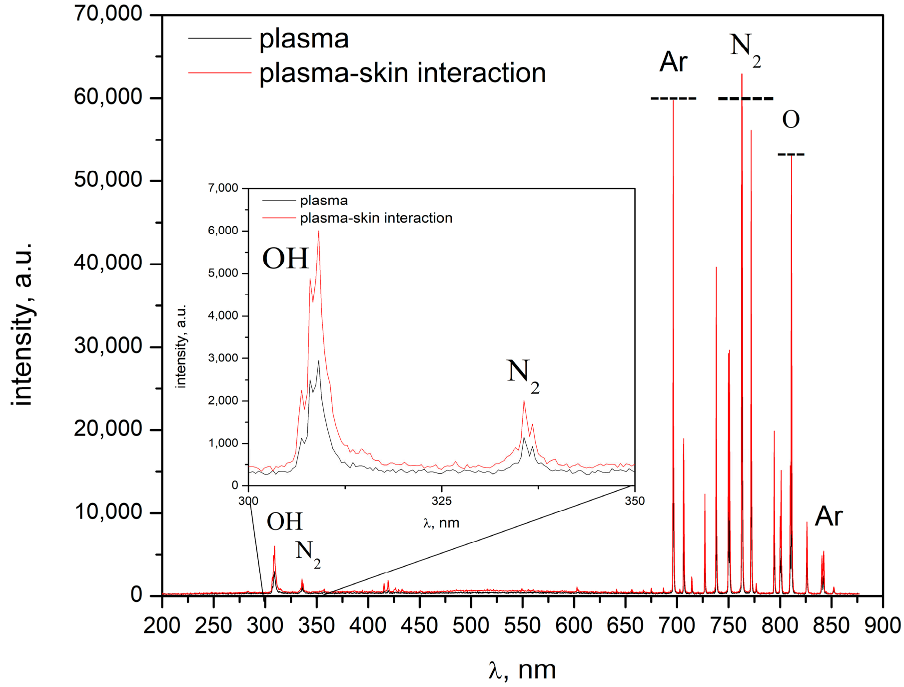


Figure 5. Optical emission spectra of an argon microwave plasma torch at the end of the torch (black line) and in contact with the skin of the mouse model (red line) at 10 W applied microwave power and 5 L/min Ar gas.

The spectral data revealed that plasma treatment enabled the efficient generation and transport of diverse reactive species into the skin, which is pivotal for therapeutic efficacy. These species are intricately involved in biochemical pathways essential for wound healing and tissue regeneration. These active particles were generated by the plasma, and without it, they would not be present near the wound site, limiting their beneficial effects on tissue repair. The presence of these species suggested that the argon plasma jet effectively interacts with the biological tissues, potentially enhancing the wound-healing process by promoting oxidative stress and stimulating cellular responses. The strong argon emission lines between 690 and 850 nm highlight the plasma's ability to maintain its activity over a significant distance, ensuring effective treatment across the targeted area.

Moreover, the consistency in the emission spectra between the plasma torch alone and the plasma torch in contact with the skin underscored the reliability and reproducibility of the plasma treatment method. This consistency is essential for ensuring that the therapeutic effects observed in controlled experimental conditions can be reliably translated into practical clinical applications.

Similar results regarding the OES of the argon plasma torch have been reported in previous studies, such as those presented in [31], further validating our findings. The ability to generate specific reactive species and maintain consistent plasma characteristics underpins the potential of this technology for various medical applications, particularly in wound healing and tissue regeneration.

The detailed analysis of the emission spectra not only provided insights into the fundamental interactions between plasma and biological tissues but also set the stage for future research aimed at optimizing plasma treatment parameters. By fine-tuning the power, gas flow, and exposure duration, it may be possible to maximize the therapeutic benefits while minimizing any potential side effects, thereby enhancing the overall efficacy and safety of plasma-based treatments.

The average wound area reduction for both the treated (MT) and non-treated (MC) groups is illustrated in Figure 6. In the group of mice treated with MCAP, a noticeable acceleration of the wound-healing process was observed compared to the untreated group. Full wound closure was observed at 4 days (for the MT group) in comparison with full closure for the MC group. The reduction in wound area showed an almost parallel trajectory between the treated and untreated groups until the shedding of the keratin layer occurred.

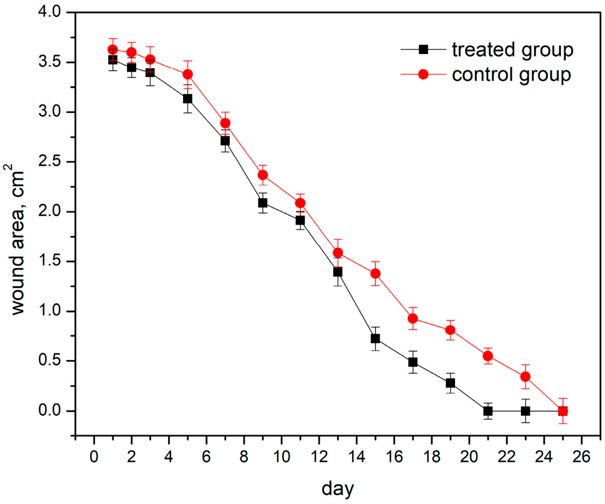


Figure 6. Wound area variations for treated (MT) and nontreated (MC) groups.

One can see the difference in the time required for the different groups to separate the keratin layer. In the MT group, separation was achieved at the end of the second week, while the MC group achieved it two days later. After detachment of the keratin layer, the reduction in wound area for both groups was parallel. However, the MT group reached complete wound closure up to 4 days before the MC group. For MT, this happened at the end of the third week or, more precisely, on the 21st day, while for the MC group, it happened on the 25th day.

This acceleration in wound healing in the treated group highlights the effectiveness of the CAP treatment. The parallel reduction in wound area post-keratin layer separation suggested that the initial stages of wound healing are critically enhanced by the CAP treatment, leading to earlier keratin layer separation and, subsequently, faster overall wound closure. These results are indicative of the MCAP device's potential to accelerate wound healing, demonstrating both its efficacy and reliability in promoting faster recovery compared to traditional methods. The accelerated healing observed in the treated group also suggested potential benefits in reducing the risk of infection and other complications associated with prolonged wound exposure, thereby improving overall outcomes in wound management.

The observed reduction in the wound area compared to the initial area of the keratin layer in our study can be attributed to the dynamic processes inherent in the wound healing cascade. As a wound progresses, several mechanisms come into play, including wound contraction, tissue remodeling, epithelialization, and granulation tissue formation. Wound contraction, facilitated by specialized cells like myofibroblasts, pulls the wound edges together, reducing the overall wound area. Tissue remodeling involves the reorganization of the extracellular matrix, resulting in a more compact tissue structure. Epithelialization, where new epithelial tissue migrates to cover the wound surface, also contributes to wound closure by reducing the visible wound area. Additionally, the formation of granulation tissue, characterized by increased vascularity and connective tissue proliferation, further aids in filling and reducing the wound area. These interconnected processes underscore the complexity of wound healing dynamics and provide a comprehensive understanding of the observed changes in the wound area relative to the surface of the initial keratin layer.

The reason for the change in the ploidy of the wound may be the dynamic processes inherent in the mechanism of wound healing. In the time after injury, several mechanisms come into play, including previously mentioned ones. Microfibroblasts facilitate changes in wound area by facilitating the contraction of its contour. Reorganization of the extracellular matrix allows for a more compact structure. This is a stage of tissue remodeling. The visible wound area is hidden by the new epithelial tissue that migrates over the wound in the epithelialization stage. The filling and reduction in the wound area are also aided by the increased proliferation of vessels and connective tissue. This occurs at the stage of granulation tissue formation. These processes are related to each other, and it is they that determine the complexity of the wound healing process. These processes are also the cause of the observed changes in the wound area relative to the surface of the original keratin layer.

Each of the processes has its own contribution to the healing of a wound. But the processes are so complex and interconnected that the difference in the contribution of individual processes to the healing of a wound should be overlooked. These differences should not be excluded between different experimental animals or under different conditions. Although the obtained results represent valuable information about the overall effect, investigating the role of the individual stages in the future should not be neglected. Such a study to determine the role of the individual stage and its change after plasma treatment would greatly contribute to the understanding of the processes.

The change in the time of the closure of the wounds was also studied together with the study of their areas. The reason for this was experimental indications of possible variations in the initial area of the wound for the different experimental animals or in the different groups. Assessment of the extent of wound healing allowed for the standardization of the study while accounting for initial differences in wound area. The results for area change were also suitable for comparison with published results from other scientific groups. This assessment allowed the detection of much smaller deviations between individual test animals and groups of animals. Wound closure is determined by the formula:

$$wound\ closure = \left(1 - \frac{area\ at\ given\ day}{initial\ area}\right) \times 100\%. \quad (1)$$

Assessment of wound closure in this study was conducted carefully, with each animal in each experimental group thoroughly examined. To ensure accurate analysis, at least six images were captured to measure the wound area for each animal. The temporal dependence of wound healing was averaged in each experimental group in order to provide a representative summary of the calculated values. In Figure 7, the averaged results are presented graphically, representing the general trends in wound closure for each group.

The observed dynamics of wound closure confirmed our initial expectations, formed by our observations of published results. Faster wound closure was observed in the treated animals, which was consistent with our expectation for the results of CAP treatment. It can be seen that this accelerated closure was in unison with the area reduction behavior in the control group, further underscoring the efficacy of the device. This correspondence between the expected and observed results could be an indicator of the credibility of our findings, reaffirming the potential of this new MCAP device as a promising therapeutic approach to improve wound healing.

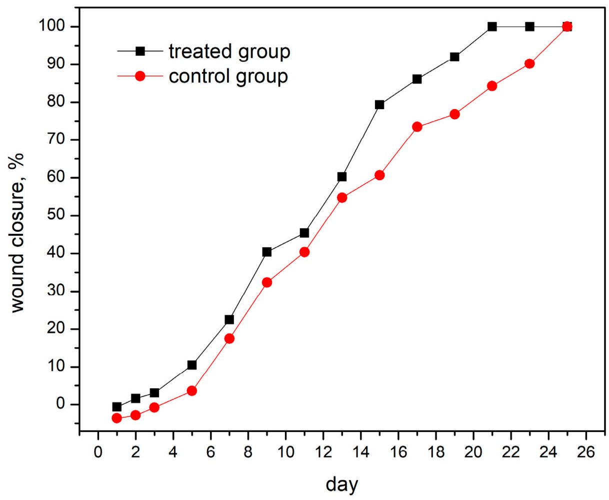


Figure 7. Average wound closure for control (MC) and treated (MT) groups.

Our study results indicated that the rate of wound closure was comparable to findings reported in studies [21,24,28], with no significant deviations observed from the outcomes reported in the study [22]. Notably, studies [21,24] also demonstrated similar periods of accelerated reduction in the wound area, particularly around the 10th-day post-injury, which corresponded to a pivotal phase in our methodology, namely the removal of the keratin layer. These similarities in results highlighted the comparability and realism of our results to other studies focusing on the effectiveness of treatment with our device. It is important to note that our study was performed in a single treatment session, distinguishing it from the continuous or repeated treatments seen in the reference articles. This difference highlighted the importance and efficacy of our chosen treatment approach, providing valuable information for its potential application in clinical research.

The presented new source of plasma shows promise as an effective means of aiding wound healing, although further research is needed to fully understand its potential effects and their mechanisms. It is well established that plasma is a source of UV radiation, and while microwave plasma does not deviate from this characteristic, our study focused on wavelengths above 300 nm, with UV spectra not explicitly investigated. Plasmas have the capacity to generate UV radiation with the energy of the UV photons depending on many factors, such as gas composition, pressure, and power input. Although some plasmas can produce UV radiation at higher energy levels, the specifics depend on the complexity of the plasma source. In the device we used, the applied microwave power did not exceed 20 W, and no evidence of long-term effects on the skin was observed. The specificity of the generated UV radiation was a function of the plasma source, but the main dependence was on the turbidity of the plasma. The presented device is not intended to operate at more than 20 W of wave power, and no indications of long-term effects on the skin have been observed in the studies conducted to date, but these should not be dismissed lightly.

It is noteworthy that the applied microwave power was directed into the gas medium through the antenna and was not directly applied to the treated area. This power was utilized for excitation and plasma sustainment, thereby being consumed by the discharge process. In known surface-wave sustained plasmas, such as the surfatron-generated ones, it has been theoretically and experimentally established that no significant electromagnetic radiation is detected outside the plasma torch. The absence of tissue heating due to microwaves, or any other source indicated the lack of a microwave heating effect. However, a comprehensive understanding of the potential effects, particularly regarding UV radiation exposure and long-term skin effects, necessitates further investigation. While our preliminary findings are promising, additional studies are needed to elucidate the full scope of the novel plasma source's impact on wound healing and its safety profile in clinical applications.

In the expansive realm of wound healing research, our study introduced a novel device with promising applications of Cold Atmospheric Plasma (CAP) for biomedical purposes. The observed acceleration of the rate of wound closure not only confirmed the efficacy of the MCAP device but warrants some detailed studies of the underlying mechanisms by which it affects wounds. Through this and our other [28,32] studies in the area of CAP, we have continued to investigate the multi-sided multichannel influence of CAP on the wound healing process.

Our results were in line with the results of previous studies, which emphasized the ability of plasma to modulate inflammatory reactions, stimulate angiogenesis, and exhibit antimicrobial properties. It is known that in healthy organisms, the healing process of a wound is standard. It is the acceleration of this process as a result of the interaction with plasma that suggests the potential of CAP to stimulate and accelerate regeneration. This is completely in line with expectations based on the existing literature on the matter.

As shown in previous studies [33,34], epidermal growth factor (EGF) plays a crucial role in the complex and dynamic process of wound healing. Although our study did not examine changes in EGF, it is important to note that this should be examined as future steps. EGF is a major regulator of cellular events, with established roles in cell proliferation, migration, and differentiation.

A significant highlight of our study lies in the innovation of a novel MCAP device. The observed enhancement in wound closure among mice treated with CAP presents an encouraging avenue for addressing the unique challenges in acute wound treatment. The presented study adds another device to the numerous ones with medical applications of CAP. A future direction of research into the applicability of the device could be its application to chronic wounds or diabetic wounds.

The research has the potential to move beyond the boundaries of wound healing and into the field of translational medicine. The demonstrated effectiveness of the MCAP device in improving wound healing suggests its potential applicability in other clinical settings, such as its application to chronic wounds, so why not in various dermatological investigations or even in surgical interventions?

4. Conclusions

In conclusion, our study represents a significant step forward in the field of wound healing research through the development and characterization of a novel microwave plasma source. The promising results obtained from our experiments in BALB-C mouse models demonstrated the potential of Cold Atmospheric Plasma (CAP) in accelerating wound closure and enhancing the healing process. Our findings not only underscored the efficacy of CAP treatment in acute wound management but also highlighted its broader implications for translational medicine.

Through meticulous analysis and interpretation of our results, we have contributed valuable insights into the mechanisms underlying CAP's therapeutic effects. Furthermore, our research opens new avenues for future investigations, particularly in exploring the role of CAP in addressing specific hurdles associated with wound care in diverse physiological conditions. Additionally, the development of the MCAP device offers exciting prospects for clinical applications beyond traditional wound management, including chronic wound care, surgical procedures, and dermatological conditions.

Overall, our study not only advances the scientific understanding of CAP but also holds promise for translating these findings into practical clinical interventions. Moving forward, further research efforts are warranted to fully elucidate the therapeutic potential of CAP and its optimal application in clinical settings.

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