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ORIGINAL ARTICLE

Evaluation of cold atmospheric pressure plasma effects on *Pseudomonas aeruginosa* wound infection in a mouse model

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ABSTRACT

Background and Objectives: Antibiotic resistance in microorganisms is a significant global health concern. Cold atmospheric plasma is an innovative and promising method for inactivating bacteria. This study aimed to evaluate the effects of cold plasma on *Pseudomonas aeruginosa* in a mouse wound infection model.

Materials and Methods: The disk diffusion method was used to perform antibiograms after isolating the bacteria. A multidrug-resistant strain was then selected. The bactericidal activity of cold helium plasma was investigated in vitro. The optimal cold plasma conditions were determined in the laboratory, with a flow of 3 liters per minute and a power of 1.1 watts. These conditions were later used for in vivo evaluations.

Results: In a laboratory study, helium gas plasma treatment for 8 minutes reduced *P. aeruginosa* by 2.5 logs. In the in vivo study, plasma reduced the wound's microbial load in mice by 1.9 log. The antibiotic treatment group had a 1.2 log reduction. Both plasma and antibiotic therapies had similar effects on microbial inactivation.

Conclusion: The overall evaluation of wound healing time and pathological features showed that plasma was generally better than antibiotic treatment. Plasma can inactivate *P. aeruginosa* in wounds and accelerate wound healing.

Keywords: Cold plasma; Multidrug-resistant; Wound healing antibacterial agents

INTRODUCTION

Treating purulent infections is now a challenge. Wound-infecting bacteria are increasingly resistant to antibiotics. Studies have shown that the most common cause of wound infections is *Pseudomonas aeruginosa*. Overuse of systemic and topical antibiotics has caused multidrug-resistant bacteria. Biofilm formation in these bacteria significantly hinders wound healing (1-3).

P. aeruginosa often infects burn patients. Infections can be endogenous, from the patient's flora, or exogenous, from the environment. The percentage of burns is directly linked to mortality from this bacterium. *P. aeruginosa* is the second most common cause of burn infections and *Staphylococcus aureus* is the most common one. Patients often survive early infections of *S. aureus*, but later face *P. aeruginosa* infections, often complicating their recovery. *P. aeruginosa* is one of the most generally isolated bacteria from surgical wounds, diabetic foot ulcers, and bedsores. Various bacteria can cause wound infections, and *P. aeruginosa* accounts for 52% of them. *P. aeruginosa* usually colonizes the deep part of the wound. This bacterium resists antimicrobials due to biofilm formation (4).

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Antibiotic resistance is increasing and causing a severe crisis in public health. The UK Department of Health estimates that, by 2050, antibiotic resistance will cause over 10 million deaths a year worldwide. Systemic antibiotics continue to play a significant role in wound care. But they can cause resistance and harm to the patient. Most systemic antibiotics have low tissue penetration. This is especially true in wounds with damaged blood vessels (5). One method is cold atmospheric pressure plasma (CAPP). It can inactivate bacteria and may have other benefits (6).

CAPP is a partially ionized gas mixture. It contains active components: charged particles (electrons, ions), neutral and excited atoms, UV photons, and reactive oxygen and nitrogen species (RONS). CAPP has many uses. They include decontamination, coagulation, and dentistry. It also treats skin diseases and cancer (6, 7). Unfortunately, we don't know how CAPP interacts with cells or tissues. However, CAPP's disinfection effects and wound healing have been reported in several studies. For the first time, Kong et al. demonstrated that cold plasma shows significant potential for inactivating bacteria under experimental conditions (8).

Although cold plasma can inactivate bacteria, it has little or no effect on mammalian cells. This difference may be due to structural differences between animal cells and prokaryotes. Additionally, researchers have used plasma in other medical fields. Plasma produces reactive species, including OH, O-, O3, nitric oxide, hydrogen peroxide, UV light, and electrons. They help to sterilize and inactivate germs. Atomic oxygen radicals exhibit strong reactivity. They are vital to plasma efficiency, especially in medicine. In medicine, these species can cause strong oxidation of cell surfaces. This method proves to be effective in inactivating bacteria (9).

Friedman's studies show that, besides their antimicrobial effect, reactive species form pores in bacterial membranes. Charged particles are essential to this process. This effect is not observed in Gram-positive bacteria. They lack an outer membrane and have a thicker peptidoglycan layer than Gram-negative. Plasma also contains ultraviolet light, which can have a germicidal effect (10). Cold plasma studies show it can kill drug-resistant bacteria in wounds and infections. This means cold plasma can eliminate these bacteria effectively. The study found that voltage, gas flow, and treatment time can affect plasma's ability to inactivate microorganisms (11).

Another study found that cold plasma can inactivate *Escherichia coli*. This finding means oxygen and toxic nitrogen radicals inactivate microbes (12). A rat study by Dobrynin showed that cold plasma could inactivate *S. aureus* in open surgical wounds. This indicates that cold plasma can inactivate bacteria in living tissue (13). Ziuzina et al. showed that cold plasma can reduce quorum sensing and biofilm formation by *P. aeruginosa*, *S. aureus*, *Listeria monocytogenes*, and *E. coli*. This study suggests that cold plasma can also affect the behavior of microorganisms (14). This study focuses on pressure plasma in treating *P. aeruginosa* wound infection, as well as evaluating the duration of wound healing and the histological changes in the mouse model.

MATERIALS AND METHODS

This study used a DBD setup to generate a cold atmospheric plasma jet (Fig. 1). The configuration employed a copper tube as the central electrode, a steel ring as the ground electrode, and a Pyrex tube as the dielectric. A high-voltage, high-frequency power supply with a sine wave generated the plasma. Helium gas was selected as the carrier gas (although Fig. 1 displays the plasma jet operating with argon gas). A mass flow meter was utilized to regulate the carrier gas flow. To create cold plasma at different settings, we used various voltages with a constant frequency of 83 kHz and different gas flows. The power consumption of the device was determined using the Lissajous method (designed at Tabriz University - Iran).

Bacterial verification and antimicrobial susceptibility testing. This study used *P. aeruginosa* iso-

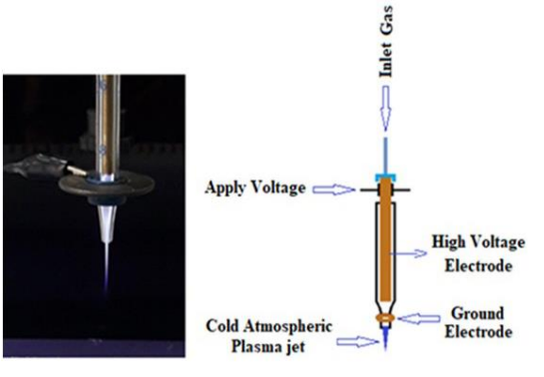

Fig. 1. Plasma jet system (left) graphic of the plasma jet system with its components (right)

lates from Tabriz University's microbial collection. Routine tests confirmed the isolates. The following tests were performed: colony morphology, pigment production on selective medium, a positive oxidase test, glucose fermentation, gelatin hydrolysis, and growth at 42°C. Then, we tested antimicrobial susceptibility using the standard Kirby-Bauer method on Muller-Hinton agar. A multidrug-resistant (MDR) strain was selected.

In vitro plasma treatment. Overall, the methodology used in the study involved culturing the isolates in nutrient broth medium overnight at 37°C with a rotational speed of 150 rpm (Labresco incubator made in-Iran), followed by centrifugation (HETTICH centrifuge / Germany) and diluted with normal saline to prepare a bacterial suspension with a concentration of 10^6 CFU ml^{-1}. The suspensions were then exposed to helium gas non-thermal atmospheric plasma for different times and characteristics, as detailed in Table 1 and 2. The results demonstrated that plasma exposure significantly reduced bacterial viability in a time-dependent manner, highlighting its potential as effective.

After the plasma treatment, the inactivation of multidrug-resistant *P. aeruginosa* was evaluated to use the plate counting method. This involved diluting the bacterial suspensions to the appropriate concentration via 10-fold serial dilutions in normal saline, and spreading 100 µL of each diluted sample on nutrient agar plates. The plates were then incubated at 37°C for 18-24 h under atmospheric conditions, and colony count was used to estimate plasma inactivation efficiency. The experiment was repeated three times.

Also, the effect of plasma gas flow and voltage was examined to find better conditions for antimicrobial plasma activity. Gas flow was investigated and confirmed in Table 1, followed by voltage changes in Table 2. The plasma produced reactive oxygen and nitrogen species. Their concentration depended on voltage, gas flow, and plasma power. The study first examined several flows and voltages in vitro. Then, it used the best conditions for in vivo experiments.

In vivo experiments in plasma treatment. In this study, 40 adult male mice weighing 25 ± 5 g were kept in individual cages under standard laboratory conditions, including room temperature, atmospheric pressure, and relative humidity of 30 ± 10%, with a 12-hour light-dark cycle and free access to food and water. The mice were divided into five groups of eight: the plasma treatment group (PTG), the antibiotic treatment group (ATG), the antibiotic control group (CGA), the plasma control group (CGP), and the non-infected wound control group (nIWCG).

Table 1. Characteristics of plasmas used for microbicide in different gas flow

He Gas Flow	Plasma power	Frequency	Voltage	
2L/min	2.2Watt	83 kHz	2.93 Kv	F1
2.5L/min	2.2 Watt	83 kHz	2.93 Kv	F2
3L/min	2.2 Watt	83 kHz	2.93 Kv	F3

Table 2. Voltage change characteristics for optimal flow

He Gas Flow	Plasma power	Frequency	Voltage	
3L/min	1.1Watt	83 kHz	2.3 Kv	W1
3L/min	2.2 Watt	83 kHz	2.9 Kv	W2
3L/min	5.2 Watt	83 kHz	4.5 Kv	W3

First, the mice were anesthetized by a peritoneal injection of Ketamine (60 mg/kg) and Xylazine (5 mg/kg). After shaving the hair, circular wounds were made on each mouse's dorsal midline. They were about 5 mm wide and 1 mm deep. The same operator did this to minimize differences in the wounds. For the wound infection model in PTG, ATG, CGP, and CGA, a 40 µl microbial suspension (OD_{600} = 2, UNICO-USA) was inoculated in sterile conditions. Then, 40 µl of sterile Nutrient Agar was poured on the wound to prepare the substrate for bacterial growth. After 72 hours, the wound infection with *P. aeruginosa* was confirmed through macroscopic and culture methods. In vivo, plasma therapy was given to the plasma treatment group (PTG) using the best in vitro result for 10 minutes (Fig. 2). Immediately after treatment, a biopsy for bacteriological study was obtained. The biopsy was placed into a sterilized pre-weighed homogenizer tube containing 1 ml of normal saline, and the tube was re-weighed. The microbial load was calculated using the following formula. On the same day, a biopsy was performed from CGP, and the microbial condition was evaluated. In this formula, C is the number of colonies. D is the dilution factor. W is the tissue weight. VN is the volume of physiological saline. VT is the volume of the inoculum.

$$c \times \mathrm{d} \times 2\frac{\mathrm{vn}}{\mathrm{w}} \times \mathrm{vt} = \mathrm{cfu/mg}$$

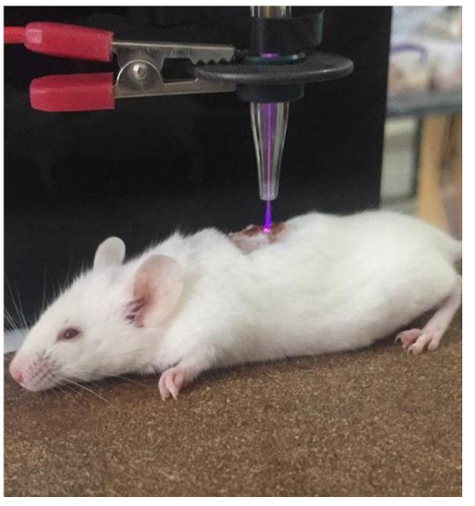

Fig. 2. Plasma treatment of mice for 10 minutes with a distance of 1 cm from the nozzle head

Antibiotic treatment. In the Antibiotic Treatment Group (ATG), two daily doses of ciprofloxacin (30 mg/kg body weight) were administered for five days. After the antibiotic treatment, a biopsy was done for a bacterial study. A similar evaluation was also conducted on the Control Group (CGA).

Pathological study of wound healing. The size of each wound was measured daily for eleven days after wound creation in the Plasma Treatment Group (PTG), Antibiotic Treatment Group (ATG), and Infection Control Groups (CGP and CGA). These groups were euthanized via peritoneal injection of Ketamine, and a 5mm diameter area of skin containing the entire wound was excised for histological examination. The tissues were fixed using routine methods, and paraffin-embedded tissues were stained with Hematoxylin and Eosin and assessed using a light microscope.

Eethics approval. All animal experiments were performed according to ethical principles and the national norms and standards for conducting medical research in Iran (Approval ID: IR.TABRIZU.REC.1400.074). All efforts were made to minimize the number of animals used and avoid undue pain.

RESULTS

A multi-drug-resistant *P. aeruginosa* strain, only sensitive to ciprofloxacin and amikacin, was selected for the plasma treatment assay. The initial *P. aeruginosa* population was approximately 6 $\log_{10}$ CFU ml^{-1}, and with increasing treatment time, the survival rate of *P. aeruginosa* gradually decreased. After 8 minutes, the surviving population was about 3.5 $\log_{10}$ CFU ml^{-1}, indicating a nearly 2.5 log reduction achieved after 8 minutes. The survival curve of *P. aeruginosa* was time-dependent, with bacterial reduction increasing linearly over time, indicating a decrease in the bacterial population with increasing plasma treatment time. The effect of helium gas flow rate (Fig. 3) and voltage (Fig. 4) on bacterial inactivation was also evaluated. The best bacterial inactivation characteristics were observed with a power of 1.1 watts, voltage of 2.3 kilovolts, and gas flow rate of 3 SLM (liters per minute), denoted as W1.

In this study, after plasma therapy was administered for infectious wounds, wound microbial evaluation showed that the microbial load of the plasma therapy group was 4.3 log and 6.2 log for CGP. The microbial load after treatment period with antibiotics was 4.4 log and 5.6 log in CGA. The variance (ANOVA) analysis of the microbial load in the plasma and antibiotic treatment groups was not statistically significant

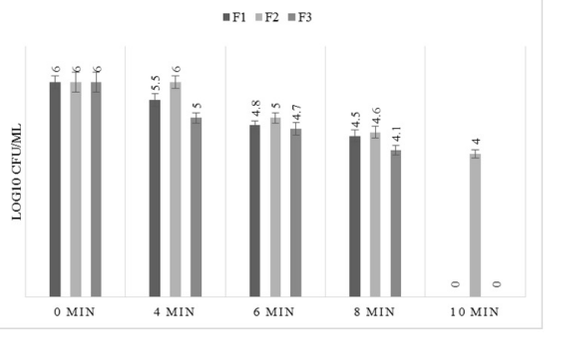

Fig. 3. Logarithmic survival data of *P. aeruginosa* after plasma exposure in different flow rates and times

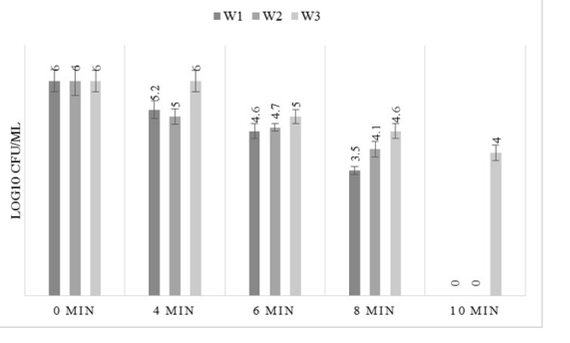

Fig. 4. Logarithmic survival data of *P. aeruginosa* after plasma exposure to different voltages and times

(Fig. 5). However, the microbial load of both treatment groups was significantly lower compared to the control group ($p<0.001$). Based on these results, the microbial reduction load in the plasma therapy and antibiotic therapy groups was 1.9 log and -1.2 log, respectively (Fig. 6).

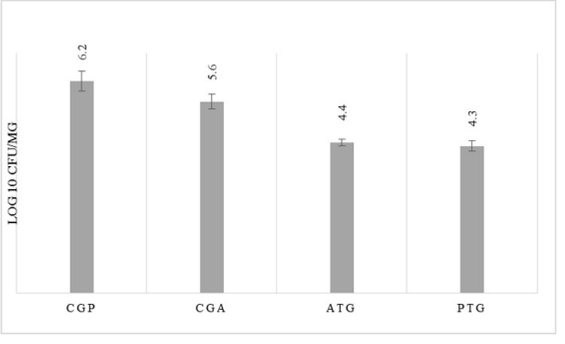

Fig. 5. Rate bacterial load of the wound in plasma treatment (PTG), antibiotic treatment (ATG) and control groups for plasma (CGP), and antibiotic (ACG)

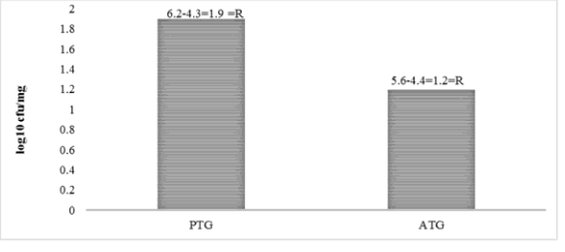

Fig. 6. Comparing the microbial load reduction of the wound with Antibiotic (ATG) and Plasma Therapy (PTG)

Evaluation of wound healing in different groups showed that wounds in the plasma treatment group closed entirely in 11 days (Fig. 7). Specimens were examined histologically on day 11. Fig. 8 shows histological microscope images of tissue samples from the untreated infection wound control group (CGP&CGA), plasma treatment group (PTG), and antibiotic treatment group (ATG). In the untreated infected groups, there was fibrosis and tissue granulation. Acute and chronic inflammatory cells were more numerous, and fibrotic tissues were delicate. In the ATG, the granulation tissue was more significant and, the fibrotic tissue was thicker, with more acute and chronic inflammatory cells than the untreated group. In the PTG, the fibrotic tissue was thicker than the untreated infectious groups but much thinner than the ATG. Additionally, there was no granulation tissue in the PTG, and acute and chronic inflammatory cells were much less. The evaluation of wound healing in different groups showed that wounds in the plasma treatment group closed entirely in 11 days.

DISCUSSION

Plasma is a rich source of reactive oxygen species, ozone, and UV radiation. Each can contribute to their germicidal properties. Besides its antimicrobial effects, cold plasma has been found to inhibit biofilm formation in bacteria, which is important. Biofilms can make bacteria more resistant to antibiotics and immune responses (15). Many studies have examined the inactivation of bacteria and spores in vitro. Mendes et al. investigated the efficacy of cold atmospheric plasma in inactivating spores of Bacillus subtilis. The results showed that spore inactivation increased linearly with increasing plasma treatment time. The authors attributed the deactivation of spores to the presence of reactive species (16). Syamas et al. conducted a study in which they reported the successful use of cold plasma to inactivate *Acinetobacter baumannii* in solid media. The findings of this study suggest that using plasma could be a promising approach to sterilizing hospital environments and medical supplies (17).

In this study, different plasma characteristics were investigated for their effectiveness in inactivating bacteria. Helium gas was used at various flow rates and treatment times, and it was found that the bacteria count decreased linearly with increasing plasma treatment time. The results also indicated that the plasma microbicide activity increased linearly as the helium gas flow rate increased, and the optimal flow rate was determined to be 3 liters per minute. Following this, the voltage effect was evaluated, and the best power was found to be 1.1 watts with the W1 characteristic. Under these optimized conditions, in vitro plasma treatment for 8 minutes resulted in a 2.5 log reduction of *P. aeruginosa*. These parameters were then applied to animal testing in vivo. It is important to note that the bactericidal effect of cold plasma can vary depending on the plasma characteristic used.

Isbary et al. conducted a study on 24 patients with chronic ulcers who received daily 2-minute treatment with cold argon plasma. The results showed a reduction in microbial load in the treatment group,

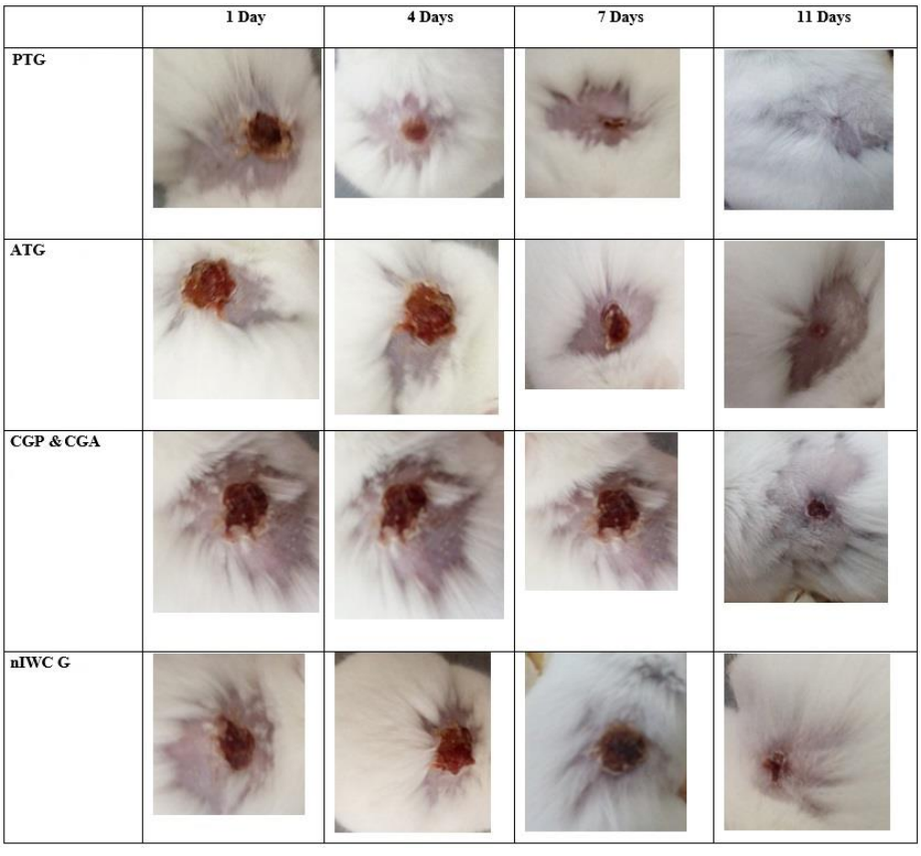

Fig. 7. Representative images of skin wounds and their contraction on days 4, 7, and 11 in control and plasma-treated groups

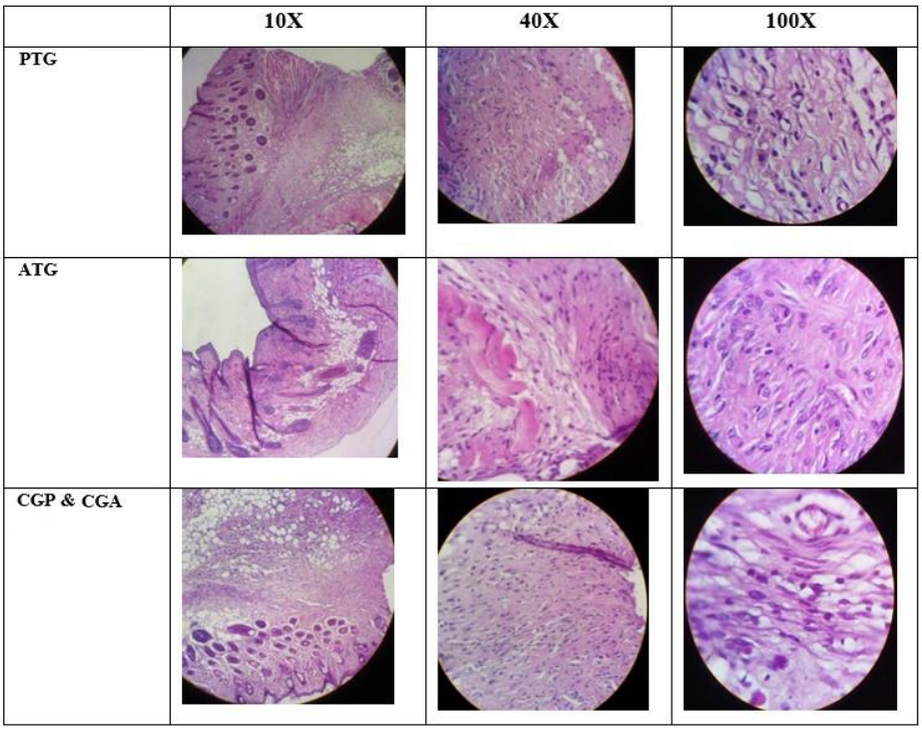

Fig. 8. Histological microscope images of wound tissue samples on day 11

regardless of the type of germ present, compared to the control group. The microbial load was assessed using a swab taken from the wound and cultured. The study also confirmed, no observed side effects in the treated patients (18).

Langner et al. showed a study to compare the effectiveness of cold atmospheric plasma with common antiseptic agents such as chlorhexidine digluconate, coctenidine dihydrochloride, and polyhexamine, to determine if plasma could be used as a supplement or an alternative to these agents for inhibiting microbial growth. The antiseptics were added to cultures containing microbes such as *P. aeruginosa*, *Streptococcus mutans*, *Candida albicans*, and *Staphylococcus epidermidis*, capable of forming a biofilm, during the planktonic stage for 32.5 hours. Plasma was applied for 60 seconds to the same bacterial cultures. The results showed that *Candida Albicans* had the highest resistance to polyhexamine, and *Streptococcus mutans* had the highest resistance to chlorhexidine digluconate. The most effective antiseptic was octenidine dihydrochloride, and plasma had similar effects to *Candida albicans* and *Streptococcus mutans* with only 60 seconds of plasma flow. The plasma effect on *P. aeruginosa* was better than its effect on Gram-positive bacteria (19). Research has shown that using antiseptic agents for disinfecting infectious wounds can delay wound healing and result in the non-migration of fibroblasts to the wound site. Fibroblasts play a vital role in the wound-healing, and their failure to migrate to the wound area can inhibit healing (20).

In our study, the Antibiotic treatment group was given two doses of ciprofloxacin daily for five days. Tissue sampling and microbial analysis were performed after the treatment period. The average microbial load in this group was estimated at 2.8×10^4 CFU mg^{-1}, and the average logarithmic of the antibiotic treatment group (ATG) was 4.4 log, which was approximately equivalent to the PTG and was not statistically significant ($p<0.001$). Simultaneous sampling was performed from the CGA, and the average microbial load in this group was 5×10^5 CFU mg^{-1}, with a Log microbial load of 5.6 log. According to our results, using a helium plasma treatment once for 10 minutes for two doses is equivalent to antibiotic treatment for five days and even more efficient than antibiotics, while also avoiding the prevalence of antibiotic resistance and various side effects. Therefore, plasma can be used as a new method for treating infected wounds. In the present study, in vitro, helium gas plasma treatment for 8 minutes reduced 2.5 log by *P. aeruginosa*. In vivo, a single 10-minute plasma treatment reduced the microbial load of the wound by 1.9 log in mice, whereas the microbial burden decreased by only 1.2 log in the antibiotic-treated group.

In the plasma-treated wounds furthermore, cold plasma has been found to enhance angiogenesis by promoting endothelial cell proliferation and migration. These effects are thought to be due to the increased production of angiogenic factors such as vascular endothelial growth factor (VEGF) and primary fibroblast growth factor (BFGF) in response to cold plasma treatment. Overall, cold atmospheric plasma has shown promising results in promoting wound healing by enhancing cell proliferation and angiogenesis (21).

In an in vitro study, Ermolaeva et al. showed that plasma treatment with argon gas on *P. aeruginosa* for 10 minutes reduced the bacterial count to 5 log (18). This study differs from our research as we observed a 2.5 log reduction in vitro, which may have been caused by differences in gas consumption. Ermolaeva et al. showed another in vivo study where they applied argon plasma gas for 5 minutes but observed no reduction in the number of *P. aeruginosa*. They evaluated microbial growth by extracting swabs from wounds and culturing them. The authors continued plasma therapy for five days, treating wound infections caused by *P. aeruginosa* and *S. aureus* for 5 minutes daily. After three days, *P. aeruginosa* was eradicated, suggesting that the duration of plasma exposure is critical for bacterial inactivation. However, on the fifth day, there was no significant difference in the amount of *S. aureus*, possibly due to structural differences between Gram-negative and Gram-positive bacteria. Furthermore, the monitoring of wound healing showed that the group infected with *P. aeruginosa* healed earlier than the *S. aureus* and control groups (22).

In another study by Xing et al., cold argon plasma with 8 kV and 1-watt power was used to heal wounds in mice. The authors treated mice with plasma for 10, 20, 30, 40, and 50 seconds daily over a 14-day period and observed that plasma treatment for less than 50 seconds resulted in faster wound healing compared to the control group. This result was confirmed by histological analysis. However, the authors also observed apoptosis and necrosis in the wound after 50 seconds of plasma treatment (23). It is possible that

differences in argon gas consumption or other variables contributed to the observed results in the study by Xing et al. In a study by Chatiraii et al., plasma treatment was performed in an animal model. The authors exposed wounds to plasma for 60 seconds daily on days 2, 3, 7, and 14 after wound creation. The results showed that the epidermal layer was produced in animals on the third day, and collagen production was increased compared to the control group. Moreover, angiogenesis increased in the plasma group by the seventh day of plasma therapy. By day 14, inflammatory cells had decreased in the plasma group, while they remained present in the control group (24). In the present study, we observed a significant reduction in inflammatory cells within the plasma group. In contrast, the untreated infectious and antibiotic groups showed increased inflammatory cells.

In another study, plasma therapy was administered daily for 14 days, with each session lasting 1 to 2 minutes. The results showed a significant decrease in wound surface area on day 7, and by day 14, the wound was closed entirely. This process was confirmed by histological results (25). Boekema et al. performed an in vitro study using a suspension of human skin cells mixed with *P. aeruginosa*, which was then subjected to argon plasma at 1.7 watts for 2 minutes. The authors found that the decrease in the bacterial count was directly related to increased plasma exposure time. Importantly, this decrease had no adverse effects on skin fibroblasts and keratinocytes (26). In another study, Boekema et al. performed an ex vivo examination of the bactericidal effect of cold plasma argon on a wound infected with *P. aeruginosa*. The authors found that within 6 minutes of plasma exposure, the microbial load was reduced to 4.5 log. Importantly, increasing the duration of plasma exposure did not have any adverse effects on fibroblasts (27). Our research differs from this study, likely due to variations in research methods and types of gas used in the plasma.

Several studies have investigated the use of cold plasma for treating chronic wounds. In Germany alone, it is estimated that 4.5 to 5 million people suffer from chronic wounds. Cold plasma has shown potential as a treatment option for managing these wounds (11). In this study, wound healing monitoring showed that wound healing occurred earlier than other groups, including ATG, (CGP&CGP), and nIWCG. Research on plasma as a microbicide and its role in wound healing is still in its early stages. However, plasma has been shown to reduce microbial burden and accelerate the wound healing process. In this study, confirmation of the rapid wound healing and closure in the PTG was obtained through pathological examination. Interpretation of the wound tissue's pathological results in the three groups showed that the PTG had less inflammation, fewer complications related to inflammation, and less scarring at the wound site. Although antibiotic use reduced the microbial burden, pathological results showed that it was ineffective in wound healing and even worsened the wound's condition. According to the results of this study, the fibrotic tissue was more aggressive in the ATG, which delayed the wound's remodeling. The fibrotic tissue was more delicate and denser in the plasma group, indicating that remodeling would occur more rapidly in the PTG. Additionally, the PTG showed significantly less granular tissue and no acute or chronic inflammatory cells. Therefore, plasma therapy is a better option than antibiotic therapy, and reducing the microbial load of the wound will have fewer side effects. Arndt et al. showed that cold plasma increased the expression of genes related to wound healing, such as IL-6, IL-8, MCP-1, TGF-1, and TGF-2. These findings suggest that plasma therapy can promote wound healing (2).

CONCLUSION

The effectiveness of plasma on bacteria indeed depends of various factors, such as the type of bacteria, gas used, plasma characteristics, and duration of exposure. However, it has been shown that cold plasma can have a general antibacterial effect without causing side effects like antibiotics. This is especially important because antibiotic resistance is becoming a growing problem. In addition to its antibacterial properties, cold plasma has also been shown to accelerate wound healing, reduce scarring, and decrease post-treatment complications. Based on the study's findings on the effects of plasma on infected wounds caused by *P. aeruginosa*, it can be concluded that cold plasma is a promising alternative to antibiotics in burn patients.

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REFERENCES

1. Hailu D, Derbie A, Mekonnen D, Zenebe Y, Adem Y, Worku S, et al. Drug resistance patterns of bacterial isolates from infected wounds at Bahir Dar regional health research laboratory center, Northwest Ethiopia. *Ethiop J Health Dev* 2016; 30: 112-117.
2. Arndt S, Unger P, Wacker E, Shimizu T, Heinlin J, Li Y-F, et al. Cold atmospheric plasma (CAP) changes gene expression of key molecules of the wound healing machinery and improves wound healing in vitro and in vivo. *PLoS One* 2013; 8(11): e79325.
3. Rijal BP, Satyal D, Parajuli NP. High burden of antimicrobial resistance among bacteria causing pyogenic wound infections at a tertiary care hospital in Kathmandu, Nepal. *J Pathog* 2017; 2017: 9458218.
4. Ferro TAF, Souza EB, Suarez MAM, Rodrigues JFS, Pereira DMS, Mendes SJF, et al. Topical application of cinnamaldehyde promotes faster healing of skin wounds infected with *Pseudomonas aeruginosa*. *Molecules* 2019; 24: 1627.
5. Engler AC, Wiradharma N, Ong ZY, Coady DJ, Hedrick JL, Yang YY. Emerging trends in macromolecular antimicrobials to fight multi-drug-resistant infections. *Nano Today* 2012; 7: 201-222.
6. Bourke P, Ziuzina D, Han L, Cullen PJ, Gilmore BF. Microbiological interactions with cold plasma. *J Appl Microbiol* 2017; 123: 308-324.
7. Hoffmann C, Berganza C, Zhang J. Cold Atmospheric Plasma: Methods of production and application in dentistry and oncology. *Med Gas Res* 2013; 3: 21.
8. Kong MG, Kroesen G, Morfill G, Nosenko T, Shimizu T, Van Dijk J, et al. Plasma medicine: An introductory review. *New J Phys* 2009; 11: 115012.
9. Han L, Patil S, Boehm D, Milosavljević V, Cullen PJ, Bourke P. Mechanisms of inactivation by high-voltage atmospheric cold plasma differ for *Escherichia coli* and *Staphylococcus aureus*. *Appl Environ Microbiol* 2015; 82: 450-458.
10. Fridman G, Brooks AD, Balasubramanian M, Fridman A, Gutsol A, Vasilets VN, et al. Comparison of direct and indirect effects of non-thermal atmospheric-pressure plasma on bacteria. *Plasma Process Polym* 2007; 4: 370-375.
11. Mohd Nasir N, Lee BK, Yap SS, Thong KL, Yap SL. Cold plasma inactivation of chronic wound bacteria. *Arch Biochem Biophys* 2016; 605: 76-85.
12. Ercan UK, Smith J, Ji H-F, Brooks AD, Joshi SG. Chemical changes in nonthermal Plasma-Treated N-Acetylcysteine (NAC) solution and their contribution to bacterial Inactivation. *Sci Rep* 2016; 6: 20365.
13. Dobrynin D, Wasko K, Friedman G, Fridman A, Fridman G. Cold plasma sterilization of open wounds: Live rat model. *Plasma Med* 2011; 1: 109-114.
14. Ziuzina D, Boehm D, Patil S, Cullen PJ, Bourke P. Cold plasma inactivation of bacterial biofilms and reduction of quorum sensing regulated virulence factors. *PLoS One* 2015; 10(9): e0138209.
15. Kim PY, Kim YS, Koo IG, Jung JC, Kim GJ, Choi MY, et al. Bacterial inactivation of wound infection in a human skin model by liquid-phase discharge plasma. *PLoS One* 2011; 6(8): e24104.
16. Mendes-Oliveira G, Jensen JL, Keener KM, Campanella OH. Modeling the inactivation of *Bacillus subtilis* spores during cold plasma sterilization. *Innov Food Sci Emerg Technol* 2019; 52: 334-342.
17. Svarnas P, Spiliopoulou A, Koutsoukos P, Gazeli K, Anastassiou ED. *Acinetobacter baumannii* deactivation by means of DBD-Based Helium Plasma Jet. *Plasma* 2019; 2: 77-90.
18. Isbary G, Heinlin J, Shimizu T, Zimmermann JL, Morfill G, Schmidt HU, et al. Successful and safe use of 2 min cold atmospheric argon plasma in chronic wounds: Results of a randomized controlled trial. *Br J Dermatol* 2012; 167: 404-410.
19. Langner I, Kramer A, Matthes R, Rebert F, Kohler C, Koban I, et al. Inhibition of microbial growth by cold atmospheric plasma compared with the antiseptics chlorhexidine digluconate, octenidine dihydrochloride, and polyhexanide. *Plasma Process Polym* 2019; 16: 1800162.
20. Turner KH, Everett J, Trivedi U, Rumbaugh KP, Whiteley M. Requirements for *Pseudomonas aeruginosa* acute burn and chronic surgical wound infection. *PLoS Genet* 2014; 10(7): e1004518.
21. Xiong Z (2018). Cold Atmospheric Pressure Plasmas (CAPs) for Skin Wound Healing. In: Plasma Medicine - Concepts and Clinical Applications. https://www.intechopen.com/chapters/60721
22. Ermolaeva SA, Varfolomeev AF, Chernukha MY, Yurov DS, Vasiliev MM, Kaminskaya AA, 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: 75-83.
23. Xu G-M, Shi X-M, Cai J-F, Chen S-L, Li P, Yao C-W, et al. Dual effects of atmospheric pressure plasma jet on skin wound healing of mice. *Wound Repair Regen* 2015; 23: 878-884.

24. Chatraie M, Torkaman G, Khani M, Salehi H, Shokri B. In vivo study of non-invasive effects of non-thermal plasma in pressure ulcer treatment. *Sci Rep* 2018; 8: 5621.
25. Kubinova S, Zaviskova K, Uherkova L, Zablotskii V, Churpita O, Lunov O, et al. Non-thermal air plasma promotes the healing of acute skin wounds in rats. *Sci Rep* 2017; 7: 45183.
26. Boekema BKHL, Hofmann S, Van Ham BJT, Bruggeman PJ, Middelkoop E. Antibacterial plasma at safe levels for skin cells. *J Phys D Appl Phys* 2013; 46: 422001.
27 Boekema BKHL, Vlig M, Guijt D, Hijnen K, Hofmann S, Smits P, et al. A new flexible DBD device for treating infected wounds: In vitro and ex vivo evaluation and comparison with a RF argon plasma jet. *J Phys D Appl Phys* 2016; 49: 044001.