Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Accelerating the healing of full-thickness excision wounds in mice using piezoelectric direct discharge plasma

M.A. Khalaf[1], S.A. Al-Sharqi[2] and B.M. Ahmed[1]

[1]Department of Physics, [2]Department of Biology, College of Sciences, Mustansiriyah University, Baghdad, Iraq

Article information

Article history:
Received 05 June, 2024
Accepted 10 August, 2024
Published online 28 September, 2024

Keywords:
PDDP
Wounds treatment
Floating electrode
Free radicals

Correspondence:
M.A. Khalaf
madyan.a@uomustansiriyah.edu.iq

Abstract

Piezoelectric Direct Discharge (PDD) plasma is a cold plasma with a floating electrode. It is a rapidly expanding and emerging wound treatment and tissue repair technology. The wound healing process is complex and requires advanced techniques to restore damaged skin. The current study utilized PDD plasma to heal full-thickness skin wounds in a mice model. The punch-biopsy created two circular wounds with a diameter of approximately 8 mm on the back, and three distinct groups of 12 healthy male mice, aged approximately 3 months, were utilized. The groups were exposed to a high voltage at 13 kV and a high frequency at 4.2 kHz plasma for 10 and 30 seconds, respectively. In contrast, the third group was categorized as the negative control. The findings demonstrate that PDD plasma exhibits notable efficacy in wound healing. The groups treated with plasma experienced a significantly accelerated healing process compared to the control group. The contraction percentage of healing on day 11 was 32.09% for the control group, 79.29, and 96.81% for the other two treated groups, 10 and 30 sec, respectively. The histological examination of skin tissue revealed enhanced tissue regeneration, reduced scarring due to developing angiogenesis, decreased inflammation, and improved treatment efficacy when the exposure time to plasma is increased. This method can be promising in adopting plasma PDD for being inexpensive and effective in promoting wound healing without side effects. This technology combines durability and ease of use.

DOI: 10.33899/ijvs.2024.150583.3709,

Introduction

The wound is characterized by disrupting the anatomical, cellular, and functional coherence of the underlying healthy living tissue. Physical, thermal, chemical, and immunological injuries or microbial agents can cause it (1,2). The wound-healing process is a constantly evolving and sequential series of events influenced by molecular, cellular, and extracellular matrix interactions (3). The physiological process of wound healing in mice typically unfolds through a series of interconnected stages, including coagulation, inflammation, migration, proliferation with matrix deposition, and remodeling, occurring sequentially (4,5). Recently, there has been a significant focus on non-thermal plasma technology due to its diverse range of biomedical applications, which include bacterial inactivation, blood coagulation, tumor treatment, and wound healing (6,7). Plasma, produced within a powerful electric field (8), is considered the fourth physical state, alongside solid, liquid, and gas (9). This partially ionized gaseous composition comprises ions, electrons, photons, reactive molecules, and ultraviolet radiation (UV), typically accompanied by significant heat generation (10,11). Cold Atmospheric Direct Plasma (CADP) operates under atmospheric conditions and room temperature, enabling painless in vivo applications without causing harm to the surrounding tissue (12). This plasma is pulsed, preventing excessive heating within a defined application period (13). CADP sources use the target region as a counter electrode (14). The primary technological advancement currently

being studied is the piezoelectric Direct Discharge (PDD) (15). These direct plasma sources generate relatively uniform plasmas that contain elevated levels of plasma-generated species (16). Pulsed diathermy PDD operates effectively when one electrode is a dielectric-protected powered electrode while the other grounded electrode is an active electrode made of animal skin or organs (17). It is important to note that discharge does not occur without animal skin or tissue surface (18). The utilization of PDD can be employed for the production of reactive oxygen species (ROS) (19) and reactive nitrogen species (RNS) (20). This involves the generation of H_2O_2, NO_2^-, NO_3^-, O_3, $ONOO^-$, $^{\bullet}OH$, and $O_2^{\bullet-}$, all of which play significant roles in wound healing (21). Different active species of plasma can have a substantial effect on sterilization (22) and a positive influence on blood coagulation without causing heating (23). Platelet-derived growth factor (PDGF) is one of the initial growth factors released at the wound site through platelet degranulation (24). It is subsequently emitted by monocyte-derived cells, fibroblasts, and endothelial cells during the later phases of wound healing (25). PDGF plays a crucial role in various stages of wound healing, especially in promoting granulation tissue formation within the wound by stimulating fibroblast proliferation, recruiting mesenchymal stem cells, and enhancing extracellular matrix production (26). Vascular endothelial growth factor (VEGF) plays a key role in promoting the formation of new blood vessels in wounds by initiating the process of angiogenesis (27). At a cellular level, VEGF interacts with monocyte-derived cells and endothelial cells through the VEGF receptor to promote the growth of blood vessels in granulation tissue (28). PDGF and VEGF are secreted at different stages of wound healing by various cell types, including platelets, monocyte-derived cells, fibroblasts, and endothelial cells (29).

The primary goal of this research is to utilize non-thermal plasma, known as PDD, as an effective, low-cost, and user-friendly technology for treating wounds.

Materials and methods

Ethical approve

The authors confirm that ethics approval for this study was obtained from the Research Ethics Committee at the College of Science, Mustansiriyah University (Approval No. BCSMU/1123/0001Ph) on November 1, 2023.

PDD plasma set-up

The Piezoelectric Direct Discharge (PDD) plasma was produced at ambient temperature using a homemade device. The outlet electrode was constructed from copper, while the insulator was made of Teflon. A dielectric barrier made of quartz, 1.2 mm thick with an outer diameter of 17 mm, is attached to the copper electrode. The device is powered by an AC high-voltage power supply ranging from 0 to 20 kV and a frequency range of 0 to 15 kHz. In this experiment, the voltage was set at 13 kV with a fixed frequency of 4.2 kHz. The spectrum of the plasma, determined using optical emission spectroscopy (OES) from the (PDD) plasma, was acquired using a fiber probe with an Ocean Optics Spectrometer, Flame-S-XR1. The plasma optical emissions from (PDD) were measured at a distance of approximately 1 mm between the animal skin and the dielectric (16).

Optical emission spectroscopy of PDD plasma

Figure 1 illustrates the optical emission spectroscopy (OES) of a flexible-powered electrode atmospheric-pressure plasma discharge (PDD) utilizing copper conductors. Wavelengths ranging from 200 nm to 400 nm indicate the presence of nitrogen radicals, while peaks between 425 nm and 600 nm correspond to oxygen radicals (30). To evaluate safety and thermal effects, the plasma was tested on the intact skin of a sedated mouse before wound application. The electron temperature (Te) was determined to be approximately 0.662 eV through spectral analysis and simulation. This electron temperature, calculated using the ratio method (31,32) with spectral lines from 315 nm to 337 nm and compared with the NIST database (33), significantly influences the production of reactive nitrogen and oxygen species (RNS and ROS). Elevated electron temperatures enhance the rate of active species production and increase plasma optical intensity, which can impact the intercellular environment (34).

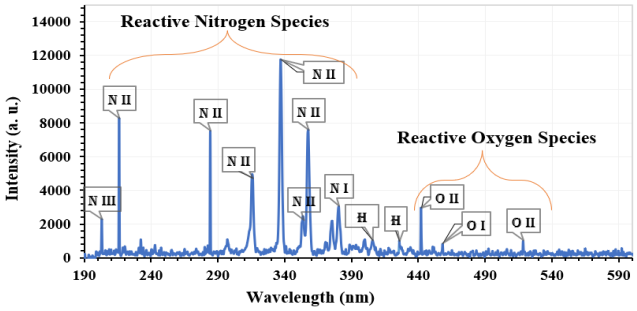

Figure 1: Optical emission spectra were obtained for atmospheric-pressure PDD with Cu conductors of the powered flexible electrode generated at 13 kV and 4.2 kHz at a distance of 1 mm from a skin surface.

Animals' preparation

Male albino mice, aged 10-12 weeks, were obtained from the Iraqi Center for Cancer and Medical Genetic Research at Mustansiriyah University in Baghdad, Iraq. The mice weighed 28 ± 5 grams and were housed in standard conditions. All the animals were kept in controlled laboratory environments that maintained atmospheric pressure, an ambient temperature of 25 ± 2 °C, and a light-dark cycle of 12 hours each. All mice had free access to water and standard laboratory food. All experiments in this research adhered to animal welfare guidelines and received

approval from the Research Ethics Committee at the College of Science, Mustansiriyah University (Reference No. BCSMU/1123/0001Ph). Additionally, the experiments adhered to the guidelines for the care and use of laboratory animals (35).

Excisional wound creation

Anesthesia was induced by administering an injection of 80 mg/kg of 10% ketamine and 10 mg/kg of xylazine to the mice that were allocated into distinct groups. Subsequently, the animals were anesthetized, and their back hair was shaved using an electric shaver followed by a razor blade. Full-thickness excision wounds involving the dermis and epidermis were created in the inter-scapular region of the animals using an 8 mm punch biopsy. No more than four mice were housed in a cage to prevent scratching and chewing. A total of 12 mice were randomly assigned to either a control group or a treatment group (36,37).

PDD plasma

The applied high voltage was 13 kV, with a constant frequency of 4.2 kHz, to generate ambient plasma on the mouse wounds. The mice were treated four times, with a dose administered every two days. The exposure times for each treatment group were as follows: the first phase involved a 10-second dose, the second phase included a 30-second dose, and the final phase consisted of a negative control group. All treatments were conducted under atmospheric pressure and at room temperature. To minimize movement during treatment, the mice were securely attached to an electrically grounded plate using adhesive tape. The wounds were carefully examined and documented multiple times, specifically on days 0, 2, 4, 6, 8, and 11 following the surgical procedure. A measuring device was positioned adjacent to each wound, and photographs were taken perpendicularly using a consistent digital camera. Notably, the photographs were consistently captured, and the mice were placed in a warm environment until they achieved complete recovery. This method is designed to be free of side effects and more efficient within a shorter time frame. The experimental investigations involved treating full-thickness skin wounds on the back by applying biopsies with varying doses of cold plasma. Macroscopic views, wound area contraction rates, PDGF, VEGF, and histological factors were evaluated. Additionally, the spectrum of plasma used in the treatment was also examined.

Mechanism of interactions reactive species with tissue

Highly reactive ions and molecules, such as oxygen, hydrogen, and nitrogen, play essential roles in many cellular and extracellular processes. Some reactive species are classified as roots, while others disintegrate to form free radicals that play a significant role in the treatment process. Reactive species vary in age and interact with various organic molecules. This difficulty in isolating these types from each other complicates the process, making it often unclear. However, their collective importance is indisputable, especially for plasma tissue treatment. When tissues (wounds) are treated with plasma, plasma generated in atmospheric gas (oxygen, hydrogen, and nitrogen) reacts with the tissue, which contains water (H_2O), to create free radicals such as ($O_2, O_2^{\cdot -}, O_2^{\cdot -2}, \cdot OH,$ and OH^-). These free radicals have covalent bonds that facilitate the transfer of electrons between the bonds, aiding in the treatment process. Highly active radicals interact with organic molecules with a typical nanosecond lifetime, a crucial example. This long period helps heal wounds or any other diseases that can be treated with cold plasma. Some free radicals, such as nitric oxide (NO), are less reactive than neutral radicals. They can diffuse through the cell membrane and facilitate signal transmission across cells. The radicals created by the plasma interaction with the tissue can generate additional reactions, such as oxygen and hydroxyl radicals, as illustrated in the equations below (38). $2O_2^- + 2H^+ \rightarrow H_2O_2 + {}^1O_2$. $O_2^- + H_2O_2 \rightarrow {}^1O_2 + OH^* + OH^-$. According to the interaction of hydroxyl radicals, hydrogen, and oxygen, enzymes are essential in reacting with them and generating ROS. $2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. $2H_2O_2 \rightarrow 2H_2O + O_2$. Upon observation, we notice the formation of radicals after the tissue is exposed to plasma. Plasma processes cells from the outside to the inside, starting with biological molecules that later signal, enhance cell membranes, and then spread through them to create noticeable effects on living organisms. The effect of plasma on tissues occurs in three layers. The initial layer of active species is created within the plasma (ROS and RNS). The second layer represents the liquid phase, where the substance transitions from the gaseous phase to the intermediate layer. The third layer, the biological medium, represents the active species that enter the cells through signaling and initiate processing (39). One of the most important activities carried out by the charges and active types resulting from non-thermal plasma, such as (OH, H_2O_2, N_2^+, O_2^-, NO, O_2, etc.), on the cell membrane through the extracellular medium is protein modification and cell reconstruction.

Sterilization by PDD plasma

Traditional methods use various antiseptic techniques before beginning wound treatment (povidone-iodine -10%). Medical contamination may result from microbes during surgical procedures or wound treatment. Toshihiro Takamatsu and his group (40) used a plasma jet mixture of helium-oxygen to demonstrate microbial studies successfully. The advantages of plasma sterilization of wounds are based on gases such as air, without relying on biological sterilizers to kill microbes, but only on ambient air atoms of nitrogen, hydrogen, etc. In current research, plasma generation for sterilizing and treating wounds relies on a single electrode with a constant frequency. PDD Plasma, particularly in the air, is highly effective and comfortable

disrupting microorganisms. PDD Plasma discharge in the air is characterized by reactive species (ROS and RNS) and can eliminate microorganisms without generating heat on the tissue and degrading them. the mechanisms of the sterilization processes for PDD Plasma, which are more sophisticated due to the significant contribution of reactive species. Consequently, the involvement of active species plays a crucial role in sterilization kinetics. Plasma sterilization is a complex process influenced by various factors determined by the plasma components, including charged and neutral particles. Each of these particles contributes differently to the sterilization process depending on their energy, speed, and the extent of interaction with the tissue. The charged particles (electrons and ions) are essential in sterilizing wounds, particularly when plasma directly interacts with microbes (38).

Histological study

After eleven days of the experiment, the mice were anesthetized. The treated wound area, approximately 2×2 mm, was excised, and the animals were euthanized by neck dislocation. Tissue samples containing the epidermis and dermis were immersed in a 10% formalin solution for 24 hours. After a series of washes and routine tissue alcohol treatments at varying concentrations, the samples were immobilized by applying paraffin wax. Thin sections measuring 5 µm were then extracted from the samples above. The slides were examined using a light microscope after staining with Hematoxylin & Eosin following the specified method (41).

Measurement of wound contraction, reduction, and epithelialization

The effects of PDD on wound healing were studied using full-thickness skin wounds in mice. The wound healing progress was evaluated by measuring the rate at which the wound contracted, indicated by the percentage decrease in area compared to its original dimensions. The wound area was measured every two days until complete closure was observed. The percentage of wound area (% wound contraction) for both the plasma group and control group was calculated (42) by considering the initial wound areas of 118.58 - 96.95 - 96.34 mm², respectively. Wound contraction= (Wound area on day 0- Wound area on day n/ Wound area on day 0)/100%. The wound area reduction ratios for different days of the three groups were calculated by determining the percentage of reduction compared to the control wound reduction ratios using a specified formula (43). Wound reduction=Wound area on day 0- Wound area on day n. The duration until closure, the number of days needed for complete epithelialization, can be calculated using a specified formula (44). Wound epithelialization=Wound area of reduction- Wound area of contraction. The measurements of the wound area were conducted using the ImageJ software.

Epithelialization time

The duration of complete epithelialization was calculated as the period during which the dead tissue scab separated from the wound area (45).

Statistical analysis

The mean ± standard deviation (SD) was used to express the quantitative data, and statistical comparisons were conducted using Microsoft Excel 2019. Significant differences were identified by a P-value of less than 0.05, and a one-way ANOVA was employed.

Results

Treatment of skin wounds in vivo

We conducted a comparative analysis of the wound areas and standard deviations between the groups that underwent plasma treatment and the untreated wounds, which served as a control group, at various time points, specifically on days 0, 2, 4, 6, 8, and 11, as illustrated in table 1.

Table 1: Displays the wound area (mm²)

Group	Day 0	Day 2	Day 4	Day 6	Day 8	Day 11
Control	^{a}96.34±2.6	^{ab}87.40±6.98	^{ab}82.15±4.10	^{ab}74.97±5.10	^{ab}70.35±3.48	^{b}65.43±1.72
10 Sec	^{a}96.72±2.29	^{a}80.56±5.11	^{c}55.03±4.08	^{cd}36.75±3.43	^{d}21.56±3.24	^{de}20.03±0.91
30 Sec	^{a}118.58±3.34	^{a}109.95±2.91	^{c}75.27±2.38	^{d}25.42±1.81	^{de}14.60±2.60	^{ef}4.02±0.87

The representative photographs of a treated wound and a control wound are illustrated in figure 2. It was observed that a scab started forming on the treated wound on day 2, while the control wound remained inflamed. The presence of a scab on the control wound persisted until day 8. No adverse effects were observed on the treated wound or its immediate vicinity. As depicted in figure 2, the ratios of wound area reduction were greater than those of the control wounds on all days. On the 6th day, the reduction ratio for the treated wounds exceeded that of the other days. The reduction ratio for the treated wound reached 96.81% and 79.29% on day 11, whereas the control wound was only 32.09% closed. When assessing the outcomes related to the disparity among the cohorts regarding duration, no significant difference was observed between the cohorts on the first day. However, a noticeable difference was identified in the treated wound. On the eleventh day, a statistically significant difference was identified. Both the application of the treatment for 30

seconds and the application for 10 seconds topically on the excision wound model resulted in a substantial increase in the rate of wound contraction compared to the negative controls. The onset of action varied, as illustrated in table 2. To investigate the correlation between the extent of wound healing and the degree of wound epithelialization. The table shows the progression of wound reduction and wound epithelialization and correlation curves at various time intervals (Tables 3 and 4). Table 4 demonstrates a significant decrease in the period of epithelialization in groups receiving treatment compared to the control group.

The therapeutic role of PDGF and VEGF in treating wounds

The expression of growth factor genes is affected by plasma treatment, as depicted in figure 3. The results revealed that on day 11, PDGF and VEGF expression levels were significantly higher in the group treated with plasma compared to the control group. Therefore, the expression levels of PDGF and VEGF were evaluated to assess the impact of plasma on wound healing, which is intricately regulated by growth factors. The experimental group subjected to plasma treatment exhibited elevated expression levels of all growth factors examined compared to the control group. This augmented expression was particularly evident on day 10, with PDGF showing upregulation by 157.012, 346.685, and 539.093, and VEGF by 34.967, 74.540, and 109.172, respectively. Angiogenesis, a critical wound-healing process, was more pronounced in the plasma-treated group. This was evidenced by increased blood vessel formation compared to the control group. This observation aligns with the increased expression of PDGF and VEGF.

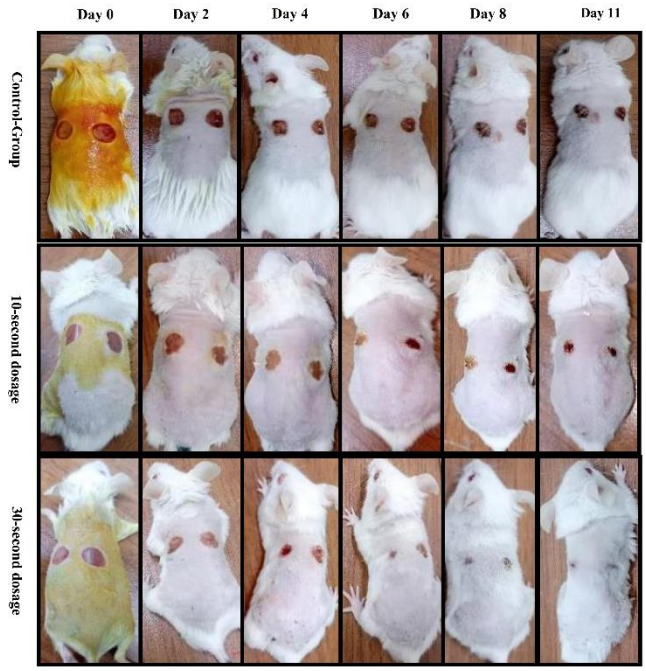

Figure 2: Macroscopic view of wound repair at different post-wounding days 0, 2, 4, 6, 8, and 11 in excision wounds of mice for the control group and the plasma treatment groups.

Table 2: Display the wound area contraction

Group	Day 2	Day 4	Day 6	Day 8	Day 11
Control	^{a}9.29±1.97	^{ab}14.73±3.89	^{ab}22.19±5.28	^{ab}26.98±3.84	^{ab}32.09±1.54
10 Sec	^{a}16.71±5.01	^{b}43.10±2.59	^{b}62.00±3.46	^{bc}77.71±3.28	^{bc}79.29±1.05
30 Sec	^{a}7.28±2.46	^{b}36.52±2.58	^{c}78.56±1.54	^{cd}87.69±2.25	^{d}96.61±0.88

The data was presented as the mean ± standard deviation, with a significance level of P<0.05.

Table 3: Display the ratios of wound area reduction

Group	Day 2	Day 4	Day 6	Day 8	Day 11
Control	^{a}8.95±2.68	^{ab}14.20±4.10	^{ab}21.38±5.54	^{ab}26.00±3.48	^{ab}30.92±1.72
10 Sec	^{a}16.16±5.09	^{b}41.69±4.69	^{bc}59.97±3.44	^{c}75.16±3.42	^{c}76.69±0.92
30 Sec	^{a}8.63±2.91	^{b}43.30±2.39	^{c}93.15±1.81	^{c}103.98±2.60	^{cd}114.56±0.78

The data was presented as the mean ± standard deviation, with a significance level of P<0.05.

Table 4: Display the ratios of wound area epithelialization

Group	Day 2	Day 4	Day 6	Day 8	Day 11
Control	^{a}0.34±0.11	^{a}0.54±0.24	^{a}0.81±0.34	^{a}0.99±0.33	^{a}1.17±0.38
10 Sec	^{a}0.55±0.18	^{a}1.41±0.29	^{ab}2.03±0.59	^{ab}2.55±0.51	^{ab}2.60±0.31
30 Sec	^{a}1.35±0.48	^{b}6.78±0.98	^{c}14.59±1.80	^{d}16.29±1.40	^{d}17.95±1.10

The data was presented as the mean ± standard deviation, with a significance level of P<0.05.

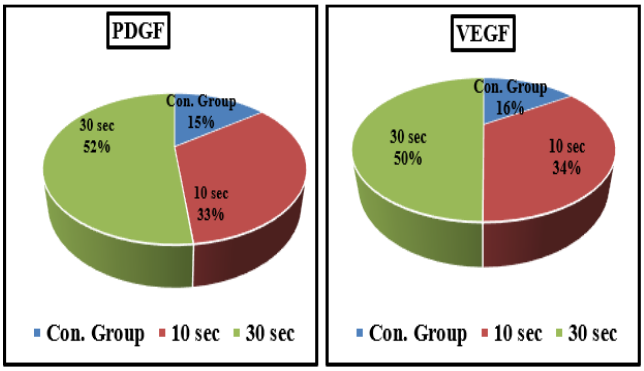

Figure 3: Levels of PDGF and VEGF at the end of wound healing were presented as median values.

Histological changes in the skin

Histological analysis of the excision biopsy specimens across experimental groups was examined using H&E staining. Figure 4 displays the control group (positive control group), illustrating the organized layers of the normal skin, including the epidermis and dermis, and the presence of hair follicles and sebaceous glands. On the 11th day post-injury, the control group (negative control group) exhibited a higher abundance of acute inflammatory cells alongside the presence of newly formed blood vessels. Assessment of the histological scores indicated incomplete progression of wound healing. Moreover, the collagen fibers within the dermal layer appeared slender and exhibited a loosely organized configuration with no complete sebaceous gland, as illustrated in figure 5.

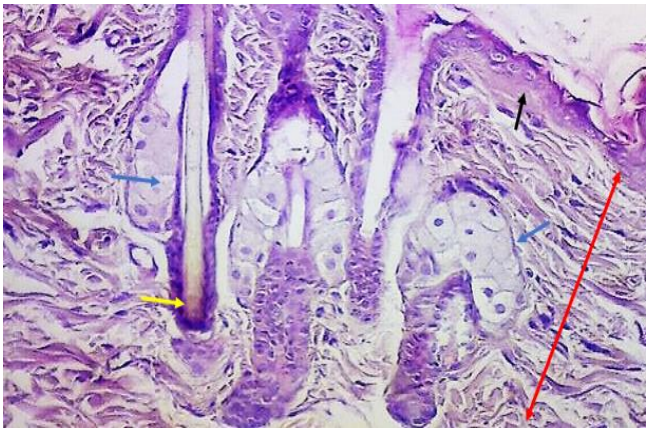

Figure 4: Longitudinal section of normal mice skin (positive control group) showing the surface of the epidermis appears regular (black arrow) and normal structure of dermal layer (double red arrow), hair follicles (yellow arrow) with associated sebaceous glands (blue arrows) (Hematoxylin and Eosin staining, 10x).

In contrast, the treatment group exposed to PDD-Plasma for 10 seconds showed reduced granulation accompanied by a moderate level of inflammation and the emergence of newly developed epidermal rete ridges, as shown in figures 6 and 7. Within the dermis, there were immature collagen fibers and increased fibroblasts. The group treated with a dose of 30 seconds exhibited a notable acceleration in epithelial regeneration alongside healed skin structures characterized by a marked reduction in inflammatory cells. Furthermore, there was complete epithelialization of the epidermal layer, accompanied by the restoration of a thin keratin layer, an elevated count of neovascularization, enhanced formation of collagen fibers, and well-developed sebaceous glands. Simultaneously, the recently generated hair follicles are observable in figures 8-11.

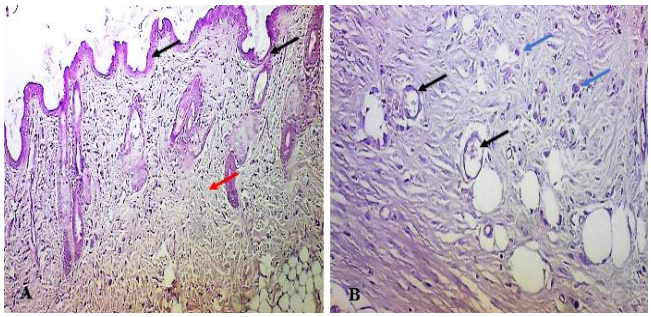

Figure 5: Longitudinal section of normal mice skin after wound healing (negative control group) on the 11th day showing (A): the surface of the epidermis appears irregular (black arrows) with the disorder of the dermal layer (red arrow), (B): new blood vessels (black arrow) and inflammatory cells infiltration (blue arrows) (Hematoxylin and Eosin staining, A: 4x, B: 10x).

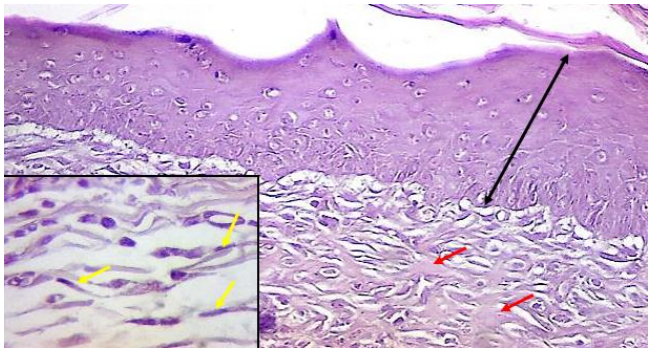

Figure 6: Longitudinal section of mice skin after wound healing on the 11th day treated by PDD-Plasma for 10 seconds showing hyperplasia of the epidermal cells (double black arrow) with increased collagen deposition (red arrow) with fibroblasts (yellow arrow) of the dermal layer (Hematoxylin and Eosin staining, large figure: 10x, small figure 40x).

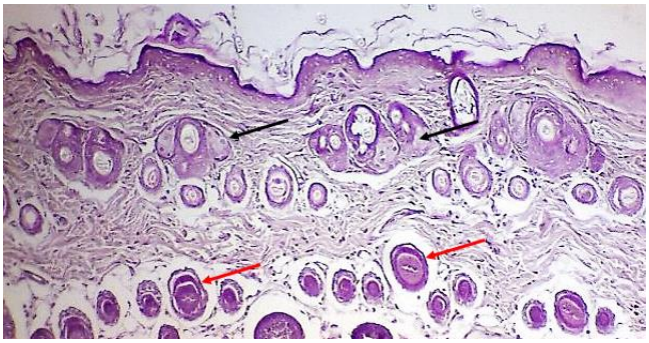

Figure 7: Longitudinal section of mice skin after wound healing on the 11th day treated by PDD-Plasma for 10 seconds showing the new formation of sebaceous glands (black arrows) and hair follicles (red arrows) in different stages (Hematoxylin and Eosin staining, 4x).

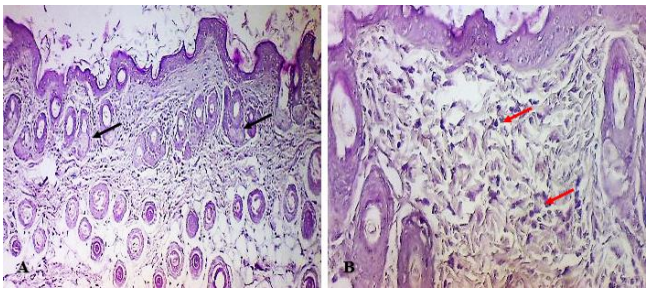

Figure 8: Longitudinal section of mice skin after wound healing on the 11th day treated by PDD-Plasma for 30 seconds showing (A): new formation of sebaceous glands (black arrows) and new hair follicle which appeared. (B): infiltration of inflammatory cells (red arrows) with hair follicles (Hematoxylin and Eosin staining, A: 4x, B: 10x).

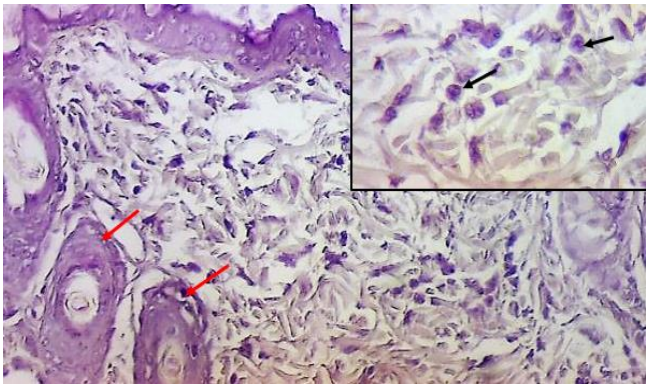

Figure 9: Longitudinal section of mice skin after wound healing on the 11th day treated by PDD-Plasma for 30 seconds showing new hair follicles which appeared (red arrows) and infiltration of inflammatory cells (black arrows) (Hematoxylin and Eosin staining, large figure: 4x, small figure: 40x).

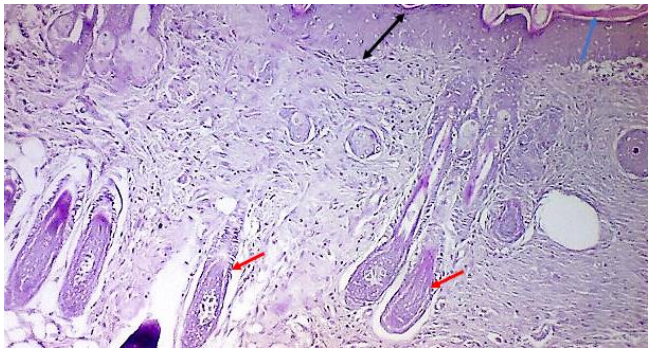

Figure 10: Longitudinal section of mice skin after wound healing on the 11th day treated by PDD-Plasma for 30 seconds showing epidermal cell regeneration was completed (black double arrow), and keratin layer was visible (blue arrow) with mature differentiation of hair follicles (red arrows) and increased collagen deposition with fibroblasts proliferation (Hematoxylin and Eosin staining, 4x).

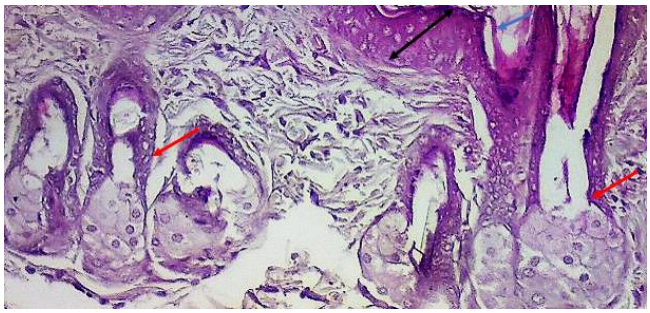

Figure 11: Longitudinal section of mice skin after wound healing on the 11th day treated by PDD-Plasma for 30 seconds showing epidermal cell regeneration was completed (black double arrow), and keratin layer is visible (blue arrow) with new formation of hair follicles and sebaceous glands (red arrows) (Hematoxylin and Eosin staining, 10x).

Discussion

Wound healing involves a series of biological and biochemical events, including blood clotting, inflammation, cell growth, movement, and specialization. These phases ultimately result in the development of granulation tissue, wound contraction, and the final restoration and remodeling of tissue. Wound contraction is the primary mechanism for wound healing in rodents. The findings of this investigation indicated an accelerated rate of wound contraction and shortened healing duration in animals in the control group. The accelerated progress in wound healing within a 30-second timeframe may be attributed to interventions in one or more stages of the healing process, thereby facilitating changes in wound contraction, epithelialization duration, and wound strength (46). Three biological consequences of PDD are observed in the relevant scholarly works: the deactivation

of a wide range of microorganisms, including those resistant to multiple drugs; increased cellular proliferation and enhanced angiogenesis within a shorter period of PDD therapy; and the induction of apoptosis after a prolonged and more intense treatment regimen, mainly targeting cancerous cells (47). PDD produces reactive oxygen species (ROS) and reactive nitrogen species (RNS), which have the potential to enhance the production of pro-angiogenic factors, thereby facilitating the process of wound healing (48). The prioritization of accelerating wound healing during the initial phase following an injury is of significant importance as it helps reduce the risk of bacterial contamination during the inflammatory stage of the wound healing process (49).

ROS and RNS are central in coordinating the typical response to injury. They function as secondary messengers for numerous immunocytes and non-lymphoid cells involved in the healing process. They play a critical role in coordinating the recruitment of lymphoid cells to the injury site and in successful tissue repair (50). They also regulate angiogenesis and the development of blood vessels at the injury site to ensure optimal blood flow to the area where wound healing occurs. They operate as a defense mechanism in the host's system through phagocytes, which release a surge of pathogens in wounds, leading to their elimination (51). The mechanisms of plasma interaction with cells and living tissues are much more complex (52). It can stimulate the proliferation and movement of skin cells by modulating integrin receptors on the cell membrane or through its angiogenic effects.

Additionally, PDD is believed to induce the production of nitric oxide (NO) (53), which facilitates cell migration and the organization of endothelial cells into vessel-like structures that aid in wound neovascularization. Hence, using plasma containing NO could be a beneficial approach for managing wound inflammation and enhancing wound treatment (54). PDD treatment can be customized to suit the different phases of wound healing. The current study's findings indicate that using PDD plasma as a safe treatment approach can positively impact the healing process and enhance wound recovery due to its antiseptic properties. The study evaluated alterations in the mechanical and histological aspects of treated tissue by characterizing PDD plasma to determine the optimal power and temperature settings and adjusting input voltage and electrode distance. The results demonstrated that plasma treatment accelerated wound healing compared to natural healing processes.

Furthermore, histological examination revealed that plasma treatment supported effective re-epithelialization, angiogenesis, new hair follicle and collagen fiber formation, and inflammation control. Additionally, mechanical analysis showed that plasma treatment could enhance tissue strength and tolerance to uniaxial tensile loads. It is suggested that the generation of reactive oxygen and nitrogen species (ROS and NOS) in plasma plays a crucial role in the interaction between plasma and tissue (55). Examining the histological microscopic images showed that plasma treatment is highly beneficial in accelerating the process of wound re-epithelialization and angiogenesis (56).

The healing rate observed in the plasma-treated group was higher than that of the control group starting from day 6 onwards, and this difference reached statistical significance on day 11. Recently, there has been a growing interest among clinicians worldwide in the medical application of cold plasma to enhance the healing process of skin wounds. Numerous studies have demonstrated that the wound-healing effect of cold plasma is attributed to its antiseptic properties, including antibacterial, anti-yeast, and antifungal properties. Recent research has shown that exposure to cold plasma significantly stimulates the migration and proliferation of fibroblasts (57,58). Additionally, another study suggests that the wound-healing effect of cold plasma is associated with the downregulation of Connexin, a gap junctional protein (59,60). These findings highlight the potential of cold plasma in facilitating wound healing, thereby reducing the discomfort and complications associated with infection or delayed wound healing. In this investigation, evidence of the involvement of cold plasma in various cellular mechanisms was further obtained through functional experiments. It was observed that the proliferation of keratinocytes is enhanced in a dose-dependent manner by cold plasma, except when the number of plasma treatments was doubled for 30 seconds. The administration of plasma significantly improved the mobility of fibroblasts during a brief treatment period of 10 seconds, suggesting its role in promoting wound closure through plasma-mediated mechanisms. These outcomes support previous findings of hormesis-like effects, where shorter treatment durations result in cellular stimulation (61-63). Growth factors play a crucial role in regulating each stage of wound healing (64). The findings suggest that plasma treatment of wounds may promote angiogenesis, thereby facilitating the wound-healing process (65).

Based on the results of contraction, reduction, epithelialization, and the findings from the histological examination, there is a strong agreement and correlation among these outcomes. Specifically, on the 11th day, the percentage of closure increases in correlation with the plasma time dose. The current research suggests that cold plasma treatment enhances the phases of wound healing and notably accelerates the rate of wound closure between days 6 and 11. Wound healing is completed in approximately 11 days with this rather large area, while other studies have indicated that healing of a wound area smaller than that covered by our research takes about 14 days or more. This means that the type of plasma used has improved and reduced the number of days for wound healing. It is possible to prevent infection by microorganisms and the wound from developing into a chronic wound. (66,67). The findings indicate that exposure to plasma may prevent the reopening of wounds, potentially offering therapeutic benefits in clinical trials.

Conclusions

This research demonstrated the safe, cost-effective, and efficient utilization of a DIY Piezoelectric Direct Discharge plasma method for healing acute wounds. This study highlights the advantages of utilizing plasma for wound treatment. The findings revealed that the PDD plasma technique effectively produces reactive oxygen and nitrogen species, which are powerful agents that assist in coagulation, tissue regeneration, and sterilization of affected areas. Plasma also facilitates the delivery of active species to cells and intricate intracellular processes through various signaling pathways. The study focused on the healing properties of different plasma doses and demonstrated that exposure to floating electrode dielectric barrier discharge plasma can accelerate skin wound recovery. This approach is vital for treatment purposes. Compared to untreated wounds, those treated with plasma showed minimal inflammation after 11 days, indicating reduced levels of inflammation in the samples. The 30-second plasma dose, known for its consistent application and rapid wound-healing effects, is recommended for wound treatment.

Acknowledgments

The authors extend they're thanks to Dr. Adnan Khazal Ajil at the Iraqi Center for Cancer and Medical Genetics Research.

Conflict of interest

The authors declare that there are no conflicts of interest regarding this manuscript's publication and/or funding.

References

1. El-Shaer AJ, Abuel-atta AA, Badr HA, AlSadek DA. The combined effect of the human umbilical cord blood with chitosan scaffold on rats' full-thickness wound healing process. Iraqi J Vet Sci. 2023;37(2):391-403. DOI: 10.33899/ijvs.2022.134782.2404
2. Sharma A, Khanna S, Kaur G, Singh I. Medicinal plants and their components for wound healing applications. Future J Pharm Sci. 2021;7(53):1-13. DOI: 10.1186/s43094-021-00202-w
3. Cárdenas-León CG, Maemets -Allas K, Klaas M, Lagus H, Kankuri E, Jaks V. Matricellular proteins in cutaneous wound healing. Front Cell Dev Biol. 2022;10(24). DOI: 10.3389/fcell.2022.1073320
4. Al-Mutheffer EA, Omar RA, Ali AF. Local application benefits of DL-methionine as wound powder: A novel approach for wound healing in rabbits. Iraqi J Vet Sci. 2024; 38(3):523-529. DOI: 10.33899/IJVS.2024.143690.3267
5. Shihab TJ, Sultan AA, Atiyah AG, Alwash SW. Evaluation of histopathological and healing potentials of the full-thickness cutaneous wound for a topical ointment formulation containing extract of bark *Quercus aegilops* in mice. Iraqi J Vet Sci. 2023;37:121-128. DOI: 10.33899/ijvs.2023.137471.2685
6. Wang XF, Fang QQ, Jia B, Hu YY, Wang ZC, Yan K-P, Yin SY, Liu Z, Tan WQ. Potential effect of non-thermal plasma for the inhibition of scar formation: A preliminary report. Sci Rep. 2020;10(1064):1-10. DOI: 10.1038/s41598-020-57703-6
7. Choil EH, Uhm HS, Kaushik NK. Plasma bioscience and its application to medicine. AAPPS Bulletin. 2021;31(10):1-38. DOI: 10.1007/s43673-021-00012-5
8. Alkareem RS, Dakhil OA, Ahmed BM. Study of plasma parameters using different voltages in an Ar/O2 dielectric barrier discharge. 5th Int Conf Appl Sci icas2023, AIP Publishing. 2024;3097(1):1-8. DOI: 10.1063/5.0209480
9. Mahdi SS, Aadim KA, Khalaf MA. New Spectral Range Generations from Laser-plasma Interaction. Baghdad Sci J. 2021;18(4):1328-1337. DOI: 10.21123/BSJ.2021.18.4.1328
10. Ahmed BM, Abdulrazaq RA, Khalaf MA, Dakhil OAA. Parameters for Fe_2O_3 on staphylococcus aureus and acinetobacter baumannil. J Eng Sci Technol. 2022;17(1):0552-0562. [available at]
11. Khalaf MA. Spectroscopic Investigation of Laser-Induced Graphene Plasma. Sci J King Faisal Univ. 2021;22(1):115-118. DOI: 10.37575/b/sci/0053
12. Kazemi A, Nicol MJ, Bilén SG, Kirimanjeswara GS, Knecht SD. Cold atmospheric plasma medicine: Applications, challenges, and opportunities for predictive control. Plasma. 2024;7(1):233-257. DOI: 10.3390/plasma7010014
13. Busco G, Robert E, Hammas NC, Pouvesle JM, Grillon C. The emerging potential of cold atmospheric plasma in skin biology. Free Rad Biol Med. 2020;161:290-304. DOI: 10.1016/j.freeradbiomed.2020.10.004
14. Gjika E, Ghosh SP, Kirschner ME, Lin L, Sherman JH, Stepp MA, Keidar M. Combination therapy of cold atmospheric plasma (CAP) with temozolomide in the treatment of U87MG glioblastoma cells. Sci Rep. 2020;10(1):1-13. DOI: 10.1038/s41598-020-73457-7
15. Korzec D, Hoppenthaler F, Nettesheim S. Piezoelectric Direct Discharge: Devices and Applications. Plasma. 2021;4(1):1-41. DOI: 10.3390/plasma4010001
16. Zoubian F, Renaut N, Latrasse L. Distributed elementary ECR microwave plasma sources supplied by solid state generators for production of large area plasmas without scale limitation: Plasma density measurements and comparison with simulation. Plasma Res Express. 2021;3(2):1-10. DOI: 10.1088/2516-1067/ac0499
17. Meghrazi MA, Ying B, Schlums A, Lam E, Eskandarian L, Abbas F, Sidhu G, Mahnam A, Moineau B, Popovic MR. Evaluation of dry textile electrodes for long-term electrocardiographic monitoring. Biomed Eng. 2021;20(1):1-20. DOI: 10.1186/s12938-021-00905-4
18. Bednar T, Babusiak B, Smondrk M, Cap I, Borik S. The impact of active electrode guard layer in capacitive measurements of bio signals. Measurement. 2021;171:1-13. DOI: 10.1016/j.measurement.2020.108740
19. Albano GD, Gagliardo RP, Montalbano AM, Profita M. Overview of the mechanisms of oxidative stress: Impact in inflammation of the airway diseases. Antioxidants. 2022;11(11):1-25. DOI: 10.3390/antiox11112237
20. Heirman P, Boxem WV, Bogaerts A. Reactivity and stability of plasma-generated oxygen and nitrogen species in buffered water solution: A computational study. Phys Chem Phys Chem. 2019;21(24):12881-12894. DOI: 10.1039/c9cp00647h
21. Shen J, Zhang H, Xu Z, Zhang Z, Cheng C, Ni G, Lan Y, Meng Y, Xia W, Chu PK. Preferential production of reactive species and bactericidal efficacy of gas-liquid plasma discharge. Chem Eng J. 2019;362:402-412. DOI: 10.1016/j.cej.2019.01.018
22. Itarashiki T, Hayashi N, Yonesu A. Sterilization effect of nitrogen oxide radicals generated by microwave plasma using air. Vacuum. 2014;10:213-216. DOI: 10.1016/j.vacuum.2014.06.018
23. Kalghatgi S, Kelly CM, Cerchar E, Torabi B, Alekseev O, Fridman A, Friedman G, Clifford JA. Effects of non-thermal plasma on mammalian cells. PLoS One. 2011;6(1):1-11. DOI: 10.1371/journal.pone.0016270
24. Kim JI, Bae HC, Park HJ, Lee MC, Han HS. Effect of storage conditions and activation on growth factor concentration in platelet-rich plasma. J Orthop Res. 2020;38(4):777-784. DOI: 10.1002/jor.24520
25. Cialdai F, Risaliti C, Monici M. Role of fibroblasts in wound healing and tissue remodeling on Earth and in space. Front Bioeng Biotechnol. 2022;10:1-18. DOI: 10.3389/fbioe.2022.958381

26. Patel S, Srivastava S, Singh MR, Singh D. Mechanistic insight into diabetic wounds: Pathogenesis, molecular targets and treatment strategies to pace wound healing. Biomed Pharmacother. 2019;112(01):1-15. DOI: 10.1016/j.biopha.2019.108615
27. Zedan ZK, Al-Ameri SH. In vivo study of impact transplantation hematopoietic progenitor cells on induced cutaneous wound healing in rabbits model. Iraqi J Vet Sci. 2022;36(3):579-586. DOI: 10.33899/ijvs.2021.130949.1899
28. White MJV, Briquez PS, White DAV, Hubbell JA. VEGF-A, PDGF-BB, and HB-EGF engineered for promiscuous super affinity to the extracellular matrix improve wound healing in a model of type 1 diabetes. NPJ Regen Med. 2021;6(1):1-12. DOI: 10.1038/s41536-021-00189-1
29. Sharda D, Kaur P, Choudhury D. Protein-modified nanomaterials: emerging trends in skin wound healing. Dis Nano. 2023;18(127):1-36. DOI: 10.1186/s11671-023-03903-8
30. Loyer F, Frache G, Choquet P, Boscher ND. Atmospheric pressure plasma-initiated chemical vapor deposition (AP-PiCVD) of poly (alkyl acrylates): An experimental study. Macromolecules. 2017;50(11):4351-4362. DOI: 10.1021/acs.macromol.7b00461
31. Khalaf MA, Hmood WJ. Influence of laser energies on tin oxide nanoparticles plasma parameters prepared by Nd: YAG Laser. Sci J King Faisal Univ. 2020;21(2):203-206. DOI: 10.37575/b/sci/0009
32. NIST Standard Reference Database 78." (Version 5.10): Jun. 01, 2024. [available at]
33. Mandal M, Sarkar M, Khan A, Biswas M, Masi A, Rakwal R, Agrawa GK, Srivastava A, Sarkar A. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) in plants-maintenance of structural individuality and functional blend. Adv Redox Res. 2022;5(01):1-21. DOI: 10.1016/j.arres.2022.100039
34. Attri P, Kumar N, Park J H, Yadav DK, Choi S, Uhm HS, Kim IT, Choi EH, Lee W. Influence of reactive species on the modification of biomolecules generated from the soft plasma. Sci Rep. 2015;4(5):1-12. DOI: 10.1038/srep08221
35. Benjamin B, Overview of laboratory animal lifestyle, care, and management: a case study of albino rats. J Appl Sci Environ Manage. 2019;23(8):1431-1435. Doi: 10.4314/jasem.v23i8.4
36. Jabbar LM, Abid TA. Treatment of infected wounds by using antimicrobial blue light phototherapy. Iraqi J Vet Sci. 2024;38(2):259-266. DOI: 10.33899/ijvs.2023.137471.2685
37. Sabeel OM, Al-Hyani OH, Al-Hasan AM, Yehea KH. The effect of omental pedicle flap with platelets-rich fibrin on reconstruction of induced-urinary bladder defect in dogs. Iraqi J Vet Sci. 2024;38(3):543-554. DOI: 10.33899/ijvs.2024.145652.3386
38. Woedtke TV, Laroussi M, Gherardi M. Foundations of plasmas for medical applications. Plasma Sources Sci Technol. 2022;31(5):1-40. DOI: 10.1088/1361-6595/ac604f
39. Massella D, Argenziano M, Ferri A, Guan J, Giraud S, Cavalli R, Barresi AA, Salaün F. Bio-functional textiles: Combining pharmaceutical nanocarriers with fibrous materials for innovative dermatological therapies. Pharmaceutics. 2019;11(8):1-29. DOI: 10.3390/pharmaceutics11080403
40. Takamatsu T, Uehara K, Sasaki Y, Hidekazu M, Matsumura Y, Iwasawa A, Ito N, Kohno M, Azuma T, Okino A. Microbial inactivation in the liquid phase induced by multigas plasma jet. PLoS One. 2015;10(7):1-9. DOI: 10.1371/journal.pone.0132381
41. Al-Sharqi SA, Chaloob AK, Al-Saleem IA. Evaluation of histological changes induced by prednisolone and cyclophosphamide in some organs of male albino mice. Iraqi J Vet Sci. 2022;36(3):671-679. DOI: 10.33899/ijvs.2021.131292.1938
42. Nandanwar M, Sharma V, Karade A, Sharma A, Kansagara A, Sakhalkar U, Sidhu Y, Gupta S, Pate M, Ghorpade P. Assessment of wound healing efficacy of Growth Factor Concentrate (GFC) in non-diabetic and diabetic Sprague Dawley rats. J Diabetes Metab Disord. 2021;20(2):1583-1595. DOI: 10.1007/s40200-021-00906-z
43. 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(1):1-11. DOI: 10.1038/s41598-018-24049-z
44. Yang Y, Xie W, Li S, Sun X, Yu B, Fu H, Chen M. Splint-free line drawing model: An innovative method for excisional wound models. Int Wound. 2023;20(7):2673-2678. DOI: 10.1111/iwj.14141
45. Mulisa E, Asres K, Engidawork E. Evaluation of wound healing and anti-inflammatory activity of the rhizomes of Rumex abyssinicus J. (Polygonaceae) in mice. BMC Complement Altern Med. 2015;15(1):1-10. DOI: 10.1186/s12906-015-0878-y
46. Chhabra S, Chhabra N, Kaur A, Gupta N. Wound Healing Concepts in Clinical Practice of OMFS. J Max Oral Surg. 2017;16(4):403-423. DOI: 10.1007/s12663-016-0880-z
47. Wu Y, S. Yu, X. Zhang, X. Wang, and J. Zhang. The Regulatory mechanism of cold plasma in relation to cell activity and its application in biomedical and animal husbandry practices. Int J Mol Sci. 2023;24(8):1-14. DOI: 10.3390/ijms24087160
48. Badr G, El-Hossary FM, Lasheen FEM, Niemat ZN, Khalaf M, Salah M, Sayed LH, Abdel-Maksoud MA, Elminshawy A. Cold atmospheric plasma induces the curing mechanism of diabetic wounds by regulating the oxidative stress mediators iNOS and NO, the pyroptotic mediators NLRP-3, Caspase-1 and IL-1β and the angiogenesis mediators VEGF and Ang-1. Biomed Pharmacother. 2023;169. DOI: 10.1016/j.biopha.2023.115934
49. Landén NX, Li D, Ståhle M. Transition from inflammation to proliferation: A critical step during wound healing. Cell Mol Life Sci. 2016;73(20):3861-3885. DOI: 10.1007/s00018-016-2268-0
50. Bassoy EY, Walch M, Martinvalet D. Reactive Oxygen Species: Do They Play a Role in Adaptive Immunity?. Front Immunol. 2021;12(22):1-13. DOI: 10.3389/fimmu.2021.755856
51. Chauvin J, Judée F, Yousfi M, Vicendo P, Merbahi N. Analysis of reactive oxygen and nitrogen species generated in three liquid media by low temperature helium plasma jet. Sci Rep. 2017;7(1):1-15. DOI: 10.1038/s41598-017-04650-4
52. Li Y, Nie L, Jin S, Sun C, Lu X. The effect of plasma on bacteria and normal cells in infected wound. Oxid Med Cell Longev. 2022;2022:1-11 DOI: 10.1155/2022/1838202
53. Oh S, Seo SB, Kim G, Batsukh S, Son K H, Byun K. Poly-D,L-lactic acid stimulates angiogenesis and collagen synthesis in aged animal skin. Int J Mol Sci. 2023;24(9):1-20. DOI: 10.3390/ijms24097986
54. Chen S, Saeed AH, Liu Q, Jiang Q, Xu H, Xiao GG, Rao L, Duo Y. Macrophages in immunoregulation and therapeutics. Sign Tran Targ Ther. 2023;8,(1):1-35. DOI: 10.1038/s41392-023-01452-1
55. Bekeschus S, Woedtke T, Emmert S, Schmidt A. Medical gas plasma-stimulated wound healing: Evidence and mechanisms: Mechanisms of gas plasma-assisted wound healing. Redox Biol. 2021;46(1):1-17. DOI: 10.1016/j.redox.2021.102116
56. Veith AP, Henderson K, Spencer A, Sligar AD, Baker AB. Therapeutic strategies for enhancing angiogenesis in wound healing. Adv. Drug Delivery Rev. 2019;146(1):97-125. DOI: 10.1016/j.addr.2018.09.010
57. Tan F, Wang Y, Zhang S, Shui R, Che J. Plasma dermatology: Skin therapy using cold atmospheric plasma. Front Oncol. 2022;12:1-16. DOI: 10.3389/fonc.2022.918484
58. Abdulqader SM. Effect of different types of fertilizers on the growth and productivity of olive trees (*Olea europaea* L.) CV. Arbequina. Mesopotamia J Agric. 2024;52(1):151-169. DOI: 10.33899/mja.2024.145274.1314
59. Hanoush NH. Honey as antibacterial agent used against bacillus spp. Isolated from locally produced juice. Mesopotamia J Agric. 2023;51(4):72-85. DOI: 10.33899/mja.2023.143201.1273
60. Jung JM, Yoon HK, Jung CJ, Jo SY, Hwang SG, Lee HJ, Lee WJ, Chang SE, Won CH. Cold plasma treatment promotes full-thickness healing of skin wounds in Murine models. Int J Lower Extremity Wounds. 2023;22(1):77-84. DOI: 10.1177/15347346211002144
61. Mirpour S, Fathollah S, Mansouri P, Larijani B, Ghoranneviss M, Tehrani MM, Amin MR. Cold atmospheric plasma as an effective method to treat diabetic foot ulcers: A randomized clinical trial. Sci Rep. 2020;10(1):1-9. DOI: 10.1038/s41598-020-67232-x
62. AL-Noaimy ZA, AL-hially AA. Hydropericardium hepatitis syndrome in chickens/article review. Mesopotamia J Agric. 2022;50(1):41-51. DOI: 10.33899/magrj.2022.132871.1164

63. Mawlood AD, Taha AT. A few hematological and biochemical markers of broilers breeders males ross-308 at various hesperidin and naringin concentrations. Mesopotamia J Agric. 2023;51(4):28-38. DOI: 10.33899/mja.2023.143067.1270
64. Yamakawa S, Hayashida K. Advances in surgical applications of growth factors for wound healing. Burns Trauma. 2019;7(10):1-13. DOI: 10.1186/s41038-019-0148-1
65. Hiller J, Stratmann B, Timm J, Costea TC, Tschoepe D. Enhanced growth factor expression in chronic diabetic wounds treated by cold atmospheric plasma. Diabetic Med. 2022;39(6):1-10. DOI: 10.1111/dme.14787
66. Lou BS, Hsieh JH, Chen CM, Hou CW, Wu HY, Chou PY, Lai CH, Lee JW. Helium/Argon-generated cold atmospheric plasma facilitates cutaneous wound healing. Front Bioeng Biotechnol. 2020;30(8):1-11. DOI: 10.3389/fbioe.2020.00683
67. Dons T, Soosairaj S. Evaluation of wound healing effect of herbal lotion in albino rats and its antibacterial activities. Clin Phytosci. 2018;4(6):1-7. DOI: 10.1186/s40816-018-0065-z

تسريع التأم جروح الاستئصال كاملة السماكة في الفئران باستخدام بلازما التفريغ الكهرضغطي المباشر

مدين احمد خلف[١]، سحر عبدالهادي الشرقي[٢] و بيداء محسن احمد[١]

[١]قسم علوم الفيزياء، [٢]قسم علوم الحياة، كلية العلوم، الجامعة المستنصرية، بغداد، العراق

الخلاصة

بلازما التفريغ الكهرضغطي المباشر هي نوع من البلازما الباردة الغير حرارية ذات قطب الكهربائي العائم. إنها تقنية سريعة التوسع ولها تأثير عالي تستخدم لعلاج الجروح وإصلاح الأنسجة. تعتبر عملية التئام الجروح معقدة وتتطلب تقنيات متقدمة لاستعادة الجلد التالف. استخدمت الدراسة الحالية البلازما التفريغ الكهرضغطي المباشر لشفاء جروح الجلد كاملة السماكة في نموذج الفئران. خلقت الخزعة المثقوبة جرحين دائريين يبلغ قطر الواحد منهما حوالي ٨ ملم في الظهر، وتم استخدام ثلاث مجموعات متميزة مكونة من ١٢ فأرا ذكرا سليما تبلغ أعمارهم ثلاثة شهر تقريبا. تم تعريض المجموعات لفرق جهد عالي عند ١٣ كيلو فولت وتردد عالي عند ٤,٢ كيلو هرتز من البلازما الباردة لمدة ١٠ و٣٠ ثانية على التوالي. وفي المقابل تم تصنيف المجموعة الثالثة على أنها السيطرة السلبية. تظهر النتائج أن لبلازما التفريغ الكهرضغطي المباشر فعالية ملحوظة في التئام الجروح. شهدت المجموعات المعالجة بالبلازما عملية شفاء متسارعة بشكل ملحوظ مقارنة بمجموعة السيطرة. كانت نسبة تقلص الشفاء في اليوم الحادي عشر ٣٢,٠٩% لمجموعة السيطرة، ٧٩,٢٩ و٩٦,٨١% للمجموعتين الأخريين المعالجتين بحوالي ١٠ و٣٠ ثانية على التوالي. كشف الفحص النسيجي لأنسجة الجلد عن تجديد الأنسجة بشكل معزز، وتقليل التندب بسبب تطور تكوين الأوعية الدموية، وانخفاض الالتهاب، وتحسين فعالية العلاج. يمكن أن تكون هذه الطريقة واعدة في اعتماد البلازما التفريغ الكهرضغطي المباشر لكونها غير مكلفة وفعالة وسريعة في تعزيز التئام الجروح دون آثار جانبية. تجمع هذه التقنية بين المتانة وسهولة الاستخدام.