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# An Analysis of the Effectiveness of Cold Atmospheric Plasma Made in Iran in Regenerating Heat and Acid Burn Wounds

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## Abstract

Burns are known by the personal and social lesions. They affect 2% of the human population with considerable mortality rates. Among the different types of burns, second-degree burns damage the epidermis and dermis. In this type of burn, bacterial proliferation has been observed as a sign of improper treatment. The main causes of burn-related deaths are the burn wound infections. Moreover, antibiotic-based treatments are considered long term and recursive treatments. Hence, physics recommends the use of cold plasma. Plasma is produced with the constant supply of energy and using a mixture of air and a new gas through ionization and presence of free radicals. The cold atmospheric plasma (CAP) made in Iran is offered with a torch equipped with a switched-mode power supply that allows high controllability of various signal parameters. A total of 20 Wistar rats were classified into two groups. Following the intraperitoneal anesthesia, one side of the vertebral column was directly exposed to boiling water using a falcon tube (with a diameter of 1.5cm), whereas the other side was directly exposed to sulfuric acid (with a diameter of 1.5cm) to cause burn with equal durations. The 1-minute treatment with 20W of electricity was administered every other day for 4 and 2 weeks for the first and second groups, respectively. After slaughtering the animals, skin samples were isolated from their bodies. Half of the skin samples entered the tissue process and sectioning was conducted. The samples were stained using the H&E and Masson's trichrome methods, and they were examined or inflammation, angiogenesis, and fibrositis. The other half was also subjected to tensiometery. As compared to the other groups, a significant statistical difference was observed in the decrease in inflammation, increase in angiogenesis, and increase in epithelium thickness in the group that experienced boiling water burns and plasma treatment for 4 weeks. The research findings revealed that the CAP made in Iran accelerated wound healing.

**Keyword**: Burn, Anti Biotic, Plasma, Wound healing, Angiogenesis, Inflammation

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## 1. Introduction

Burns are an issue in today's societies as they not only cause irreversible damage to the patients, but also impose numerous other burdens on the families [1]. According to the related statistics, approximately 2.5 million Americans are burnt every year. Of this number, over 100,000 patients are hospitalized and over 10,000 patients pass away [2]. In second-degree burns, the epidermis and dermis are fully and partially destroyed, respectively. Superficial burns are accompanied by redness, wetness and fluid leakage, blisters, and intense pain and sensitivity to touch [3]. On the other hand, the control of chronic wounds is a modern stress factor, because it affects 1 to 2% of the human population. Venous wounds require 24 weeks of treatment, and approximately 15% of the patients are never treated and 71% are affected at least one or several other times [4-5]. In addition, the therapeutic support for these patients puts an onerous economic burden on governments and societies [6]. In this regard, there are various treatments for burns including venous treatment, antibiotic treatment, hyperbaric oxygen therapy, surgical therapy, and flap treatments. Colonization of the bacteria in such wounds is the common cause and it is, evidently, the cause of inefficient wound treatment and restoration. The main causes of mortality in patients suffering from burns are also burn wound infections [7-8-9]. Hence, researchers have made numerous attempts at developing these proper methods and treatments for reducing the likelihood of wound infections, and subsequently shortening the treatment term. To this end, it is of substantial important to use methods that minimize the intake of antibiotics and prevent antibiotic resistance. Physics proposes the cold atmospheric plasma (CAP) for this purpose. Plasma is the fourth state of matter resulting from the constant supply of energy in combination with air and a new gas such as argon [10]. It is the product of the ionization of atoms in air and production of plasma containing negative and positive ions, electrons, free radicals, ozone, and ultraviolet waves [11]. Although it is possible to produce plasma from heat, electricity is utilized to produce plasma in the ambient temperature. This type of plasma is known as the non-equilibrium plasma (nonthermal plasma) or cold plasma (CAP), which offers all of the properties of high-temperature plasma except for heat generation [12]. The medical studies on cold plasma have attracted attention recently. Moreover, the cold plasma made in Iran consists of a torch equipped with a switched-mode power supply with high controllability, allowing control over the various parameters of signals applied to the torch such as frequency, voltage range, and pulse width. The frequency of this power supply varies from 2.5kHz to 50kHz and its voltage ranges from several volts to 20 kV. Its pulse width is also in the range between 1% and 99%. The power supply has a maximum current capacity of 10mA. The plasma torch is composed of an aluminum nozzle with a span diameter of 6mm and a length of 3cm as well as an axial stainless steel electrode with a diameter of 3mm. The designed torch works with noble gases, air, and nitrogen.

## 2. Materials and Method

Anesthesia was induced in 20 Wistar rats through intraperitoneal injection of ketamine and xylazine. The back skin of the animals was shaved on both sides of the vertebral column. To inflict burns on the two sides of the vertebral column, two burns were created on one side using boiling water and two other burns were induced on the other side using sulfuric acid. In addition, in order to induce a burn by boiling water, 10 seconds of direct contact between the skin and boiling water were used with a 15cc flacon tube (with a diameter of 1.5cm) that contained boiling water. The sulfuric acid burn was also induced by exposing the skin to sulfuric acid on a surface with a diameter of 1.5cm for 10seconds and washing the skin with water. The rats were put into two groups: the first group received treatment for 2 weeks, whereas the second group received treatment for 4 weeks. The one-minute plasma treatments were administered every other day using 20V electricity. At the end of the treatment course, the rats received anesthesia and were killed to collect skin samples from the restored wounds and the surrounding healthy tissues using a scalpel. The tissue samples were stored in 10% formalin and were prepared through the tissue process. The sectioning and staining of the resulting samples started post-molding. The molds containing the samples were sectioned using a manual microtome. The sections were stained using the haematoxylin, eosin, and Mallory's trichrome stains to conduct a histologic study. Afterwards, images of all sections were obtained and the measurements and counts were carried out in 10 regions using Motic to study the inflammatory cell infiltration, angiogenesis, and epithelium thickness variations.

**Table 1:** The comparison of inflammation degrees in the groups with burn wounds caused by heat and acid (Mann-Whitney test*, Kruskal–Wallis test)

| Output week | | Inflammation degree | Group: Water burn+plasma | Acid burn+plasma | Water burn-control | Acid burn-control | p-value## |
|---|---|---|---|---|---|---|---|
| | 2 | SD ± Mean | 0.560±.002 | 0.68±0.002 | 0.79±.0010 | 0.68±.0010 | 0.000 |
| | | #p-value (comparison to plasma treatment) | Reference group | 0.008 | 0.008 | 0.008 | |
| | 4 | SD ± Mean | 0.42±.001 | 0.44±0.001 | .408±0.001 | 0.47±.0010 | 0.000 |
| | | #p-value (comparison to plasma treatment) | Reference group | 0.008 | 0.008 | 0.008 | |

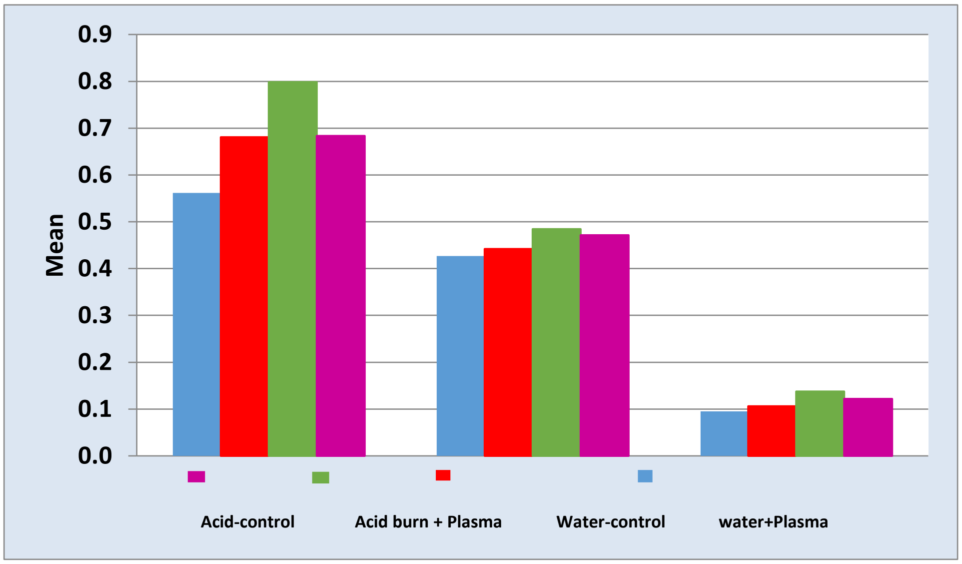


**Diagram 1:** The comparison of inflammation degrees in different plasma-treated groups in different weeks

## 3. Results and discussion

Data description and analysis was carried out in SPSS 23. The mean and standard deviation indices were used to describe the parameters of each group. Moreover, the Kolmogorov–Smirnov test was carried out to examine the normality of the distribution of the quantitative variables, viz. inflammation degree, angiogenesis degree, and epidermis thickness variations. The results from this statistical test were reflective of the non-normal distribution of all quantitative variables. Therefore, the nonparametric Kruskal–Wallis test was carried out to compare the mean values of the four groups, while the nonparametric Mann-Whitney test was carried out to compare each group with the reference group (plasma]. For all analyses, tables were used along with the comparative diagrams drawn in Excel. Table 1 and Diagram 1 present the results from the comparisons between the degrees of inflammation in the study groups based on different outputs. According to this table, in output 1, the highest degree of inflammation is observed in the boiling water burns group (with a mean of 79 0.001), whereas the lowest degree is obtained in the plasma-treated boiling water burns group (with a mean of 0.56 0.002) (figure 1-2). The results from the Kruskal–Wallis test also suggest that in the first output, there is a statistically significant difference in the degree of inflammation in the four groups (p-value=0.000). According to the comparison between the plasma-treated boiling water burns group with other groups through the Mann-Whitney test, inflammation was significant lower in this group than all the other groups (p-values<0.05). The same result was observed in the subsequent outputs and the inter-group comparisons.

The findings from the comparison between the angiogenesis results of the experimental (treatment) groups in various weeks are presented in Table 2 and Diagram 2. According to this table, in the second output week, the plasma-treated boiling water burns group showed the highest degree of angiogenesis with a mean of $4.23 \pm 0.17$. The Kruskal–Wallis test also indicated that on this day, there was a statistically significant difference in the angiogenesis results of the 4 study groups (p-value=0.007). The results from comparing different groups with the Zosh ointment group through the Mann-Whitney test revealed that angiogenesis was significantly higher in the mentioned group than the other groups except for the plasma-treated acid burns group (p-values <0.05). However, there was no significant difference between this group and the plasma-treated acid burns group (p-value=0.095).

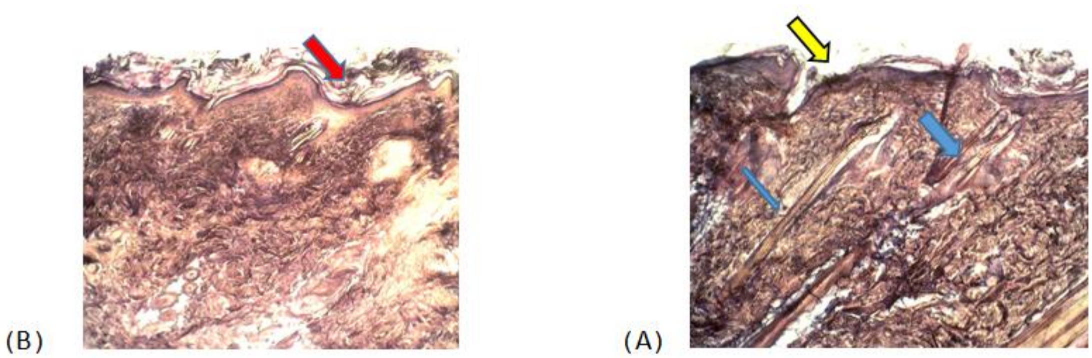


**Figure 1**: H&E staining of the skin surface depicting the complete regeneration process. In this figure, the skin is burnt by boiling water and the results from 4 weeks of treatment with plasma are depicted. No inflammation is present (yellow arrow) and the hair follicles (blue arrow) are observed. (A) This image is of the same group in the second week of plasma therapy. As indicated by the red arrow, epidermis is in the cornification phase; (B) the magnification is x100.

In the fourth output week, the highest angiogenesis was observed in the plasma-treated boiling water burns group (with a mean of 7.58 0.31), while the lowest degree of angiogenesis was observed in the burns control group (with a mean of 6.19 0.36). The results from the Kruskal–Wallis test also suggested that in this output week, there was a statistically significant difference in angiogenesis in the 4 study groups (p-value=0.005). The comparison of the study groups with the plasma-treated boiling water burns group through the Mann-Whitney test showed that angiogenesis was higher than the other groups in this group (p-values <0.05).

The comparison between the variations of epidermis thickness with different treatment types in different output weeks are presented in Table 3 and Diagram 3. The results listed in this table indicate that in the second week, the highest epidermis variations were observed in the plasma-treated boiling water burns group (with a mean of $45.80 \pm 0.52$), whereas the lowest level was observed in the boiling water burns control group (with a mean of $40.16 \pm 1.08$). According to the Kruskal–Wallis test, on the third day, there was a statistically significant difference between the variations of epidermis thickness (p-value=0.002) in the 4 groups. The results from the comparisons between the study groups through the Mann-Whitney test indicated that the epidermis thickness variations were significantly higher in the plasma-treated boiling water burns group than the other groups, except for the group burnt with acid and treated with plasma (p-values<0.05). These results did not reveal a statistically significant difference between this group and the plasma-treated acid burns group (p-value=0.095). Based on the results obtained in the fourth week, the highest level of changes of epidermis thickness was observed in the plasma-treated water burns group (with a mean of $64.81 \pm 0.29$), whereas the lowest level was observed in the acid burns group (with a mean of $62.11 \pm 0.51$). The results from the Kruskal–Wallis test also suggest that in this week, there was a statistically significant difference in the epidermis thickness changes in the four groups (p-value=0.001). The results from the comparison of different groups and the plasma-treated boiling water burns group through the Mann-Whitney test suggested that the epidermis thickness changes in this group were significantly higher than the other groups (p-values<0.05).

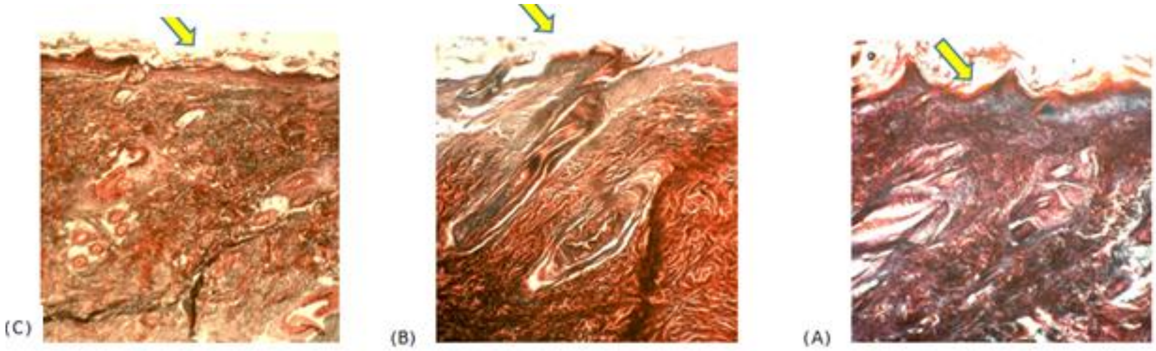


**Figure 2**: Trichrome staining of the skin surface depicting the inflammation mitigation process. In this figure, the skin is burnt by boiling water and the results from 4 weeks of treatment with plasma are depicted. No inflammation is present (yellow arrow). (A) This image is of the same group in the second week of plasma therapy. As indicated by the yellow arrow, inflammation has decreased; (B) In this figure, the inflammatory PMN cells are evident at the arrows and the image shows the early days of burning; (C) the magnification is x100.

## 4. Disscussion

In the past decades, various studies have been conducted on different types of treatments, but since the emergence of the high technology, the medical society has resorted to the applications of physics to medicine. Hence, CAP has been used in dentistry as a tooth whitener [13], for the treatment of dental plaques [14], as an alternative treatment for cancer [15-16], for sterilization and disinfection [17-18], for wound healing [19-20], and for blood coagulation [21]. Although some new studies have addressed the effect of cold plasma on wounds, most studies are focused on the medical use of the antimicrobial CAP pills [22] [23]. Tipa and Krosen stated that plasma treatment can stimulate the proliferation of fibroblast cells in the wound cell culture model and thus contribute to the wound healing [23]. Grigoras et al. [24] studied the re-epithelialization of the contaminated skin wounds in Wistar rats and stated that plasma treatment accelerates re-epithelialization. In any case, the effect of plasma on wound healing is more considerably dependent upon the target cell and tissue, radiation dosage (power and time), and voltage [12]. In medicine, high-temperature plasma is used in the sterilization of tools and equipment, tissue damage, sectioning, and cauterization. CAP offers all of the advantages of high-temperature plasma but does not generate high temperature [19]. The effectiveness of two-minute cold argon plasma in achieving a significant decrease or an extremely significant decrease in the bacterial load on the chronic infectious wounds has been demonstrated. It was also stated that the effect of plasma treatment on the bacterial load does not depend on the wounding factor and bacterium type [19]. The effect of plasma radiation on the retina of rabbits indicated that this substance has no harmful effect on the eyes. It was also reported that plasma does not considerably affect the improvements in the retina detachment within the first 24 hours, but its effectiveness in improving the wounds in longer durations has been proved [25]. In their research, Grigoras et al. [24] indicated that CAP increased the speed of re-epithelialization in the Wistar rats' skin lesions. Friedman et al. carried out studies on the tissues of human corpses and SKH1 rats to determine the maximum acceptable dosage that causes no tangible or tissue damage. They reported that the application of 0.6W per cm2 for 10 minutes and 2.3W per cm2 for 40 seconds offers the maximum plasma radiation dosages that cause no damage [21]. After 20 days of using CAP for 2 minutes, the rabbits' retinae showed no structural microscopic change. On the other hand, Bern et al. indicated that two minutes of CAP radiation did not affect the survival of keratocytes in the cellular culture, but more than 5 minutes of exposure reduced the survival of the cells [7]. According to the image-based analyses and histologic tissue staining, CAP also leaves no scar [25].

**Table 2:** The comparison of angiogenesis in different groups with burn wounds caused by heat and acid (#Mann-Whitney test, Kruskal–Wallis test)

| Angiogenesis degree | | | Group | | | | **p-value##** |
|---|---|---|---|---|---|---|---|
| | | | Water burn + plasma treatment | Acid burn + plasma treatment | Water burn – control | Acid burn – control | |
| **Output week** | 2 | SD ± Mean | 4.23±0.17 | 0.17± 4.03 | 0.47± 2.70 | 0.53± 3.67 | 0.007 |
| | | (comparison with plasma treatment) p-value | Reference group | 0.095 | 0.008 | 0.047 | |
| | 4 | SD ± Mean | 0.31± 7.58 | 0.42± 6.97 | 0.36± 6.19 | 0.31± 6.91 | 0.004 |
| | | (comparison with plasma treatment) p-value | Reference group | 0.032 | 0.008 | 0.016 | |

**Table 3**: Comparison of the epidermis thickness changes in the study groups with restoration of burn wounds caused by heat and acid (Mann-Whitney test; Kruskal–Wallis test)

| Epidermis thickness variations | | | Group name | | | | p-value |
|---|---|---|---|---|---|---|---|
| | | | Water burn + Plasma | Acid burn + Plasma | Water burn - control | Acid burn - control | |
| Output week | 2 | SD ± Mean | 45.80±0.52 | 0.79± 44.97 | 1.08± 40.16 | 2.43± 41.72 | 0.002 |
| | | Compared to plasma treatment (p-value) | Reference group | 0.095 | 0.008 | 0.008 | |
| | 4 | SD ± Mean | 0.29± 64.81 | 0.83± 63.83 | 0.39± 62.86 | 0.51± 62.11 | 0.001 |
| | | Compared to plasma treatment (p-value) | Reference group | 0.032 | 0.008 | 0.008 | |

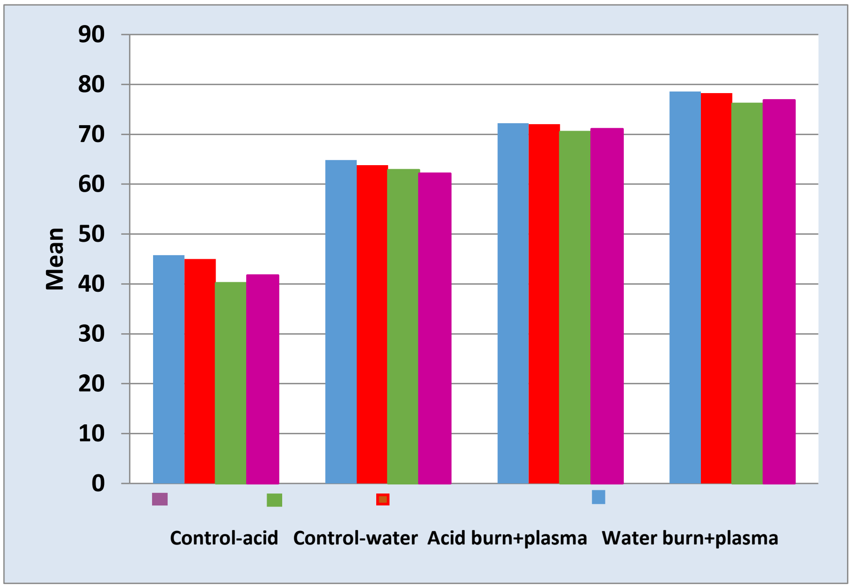


**Diagram 2**: A comparison of angiogenesis in different plasma treatments in various output weeks

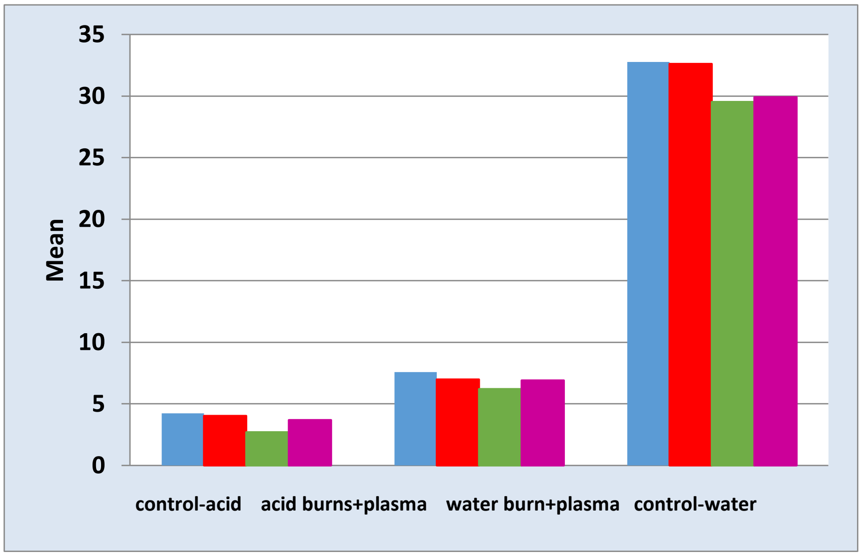


**Diagram 3:** The comparison of the epidermis thickness variations in different treatment groups in various output weeks

## 5. Conclusions

In this experiment, it was indicated that the group receiving cold atmospheric plasma for 4 weeks displayed a decrease in inflammation, an increase in angiogenesis, and an increase in the epithelium thickness.

## Conflict of Interests

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

## Acknowledgements

According to the research results, which are in line with the findings from the similar studies, it could be concluded that cold plasma is an effective treatment. However, other therapeutic courses and dosages must also be tried and studied to see whether the results are positive or reversed. Finally, CAP can be employed a complementary treatment with other common treatments as to study the synergetic effects.

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