*Journal Basic Science and Technology 13 (2) (2024) pp. 49-55*

Published by: IOCSCIENCE

Journal Basic Science and Technology

Journal homepage: www.iocscience.org/ejournal/index.php/JBST

# The important role of cold atmospheric plasma in wounds healing

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**A R T I C L E I N F O**

***Article history:***

Received May 12, 2024
Revised June 18, 2024
Accepted June 30, 2024

***Keywords:***

Cell migration;
Cold plasma;
Fibroblasts;
RONS;
Wound healing.

**ABSTRACT**

Cold plasma is the fourth state of matter it can be generated using different types of gases like argon or helium or other gases by applying electrical field to get plasma with a temperature lower than 40degree so it's considered safe to be used in human without affecting the normal body cells. Recently cold plasma came to be known as one of the most important technologies in medicine and pharmaceutical sciences. In this study we will be focusing on discussing wounds, wounds healing, different methods that help in wound care and the important role of cold plasma in accelerating the wound healing process by reviewing all the recent studies in this subject to offer a full understanding, this study showed the cold plasma has the ability to improve and accelerate wounds healing through specific mechanisms which help in cell migration and proliferation in addition to increase the vascularization at the wound side and help in gathering the fibroblasts and keratinocytes and the production of reactive nitrogen and oxygen species (RNS,ROS) and this encourages the future researches and in-vevo studies to prove its efficiency in other medical fields and make use of its many effects like antiseptic, antimicrobial, anti-tumor and also its many application in dental field like endodontic treatment, antibacterial in dental caries and implants.



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## 1. INTRODUCTION

The emergence of plasma was in the 1879 by William Crookes who first applied a high voltage to an ionized gas and called it the radiant gas. Later Irvin Langmuir worked on this technique and the term plasma was used (Darmawati et al., 2019) plasma is the fourth state of matter which is made of ionized gases partially or entirely. Its generated by apply electrical filed on a neutral gas which resulting in irradiation or excitation (Lu et al., 2016), (Choudhury et al., 2020))

Non- thermal plasma or cold atmospheric plasma is mainly an ionized gases that contain a very high amount of reactive chemicals like (ROS: reactive oxygen species) or (RONS: reactive nitrogen species), charged particles like ($H_2O_2$, e- and OH-), UV photons and also excited molecules (Choudhury et al., 2020)

Different factors affect the physical and chemical characteristics of cold atmospheric plasma which include the applied power, pressure, atmosphere and the type of gas/ gas mixture used . Cold plasma played a vital role in many medical and biological applications due to its low temperature as

well as its antibacterial effect because it causes great reduction in bacterial count in wound and improves the healing process(Niedźwiedź, Waśko, Pawłat, & Polak-Berecka, 2019).

The cold atmospheric plasma is used in human by two different approaches. The first approach is the plasma based method (the indirect method) which is done by using plasma in treating materials, surfaces or devices and then using them in treatment while the direct method is done by applying the physical cold plasma directly on animals or humans to make use of its therapeutic properties, this therapeutic properties were studied in many different articles and it was found to be very effective therapy in many medical fields ((Bernhardt et al., 2019), (Dubuc et al., 2018)).

The aim of this review is to focus on the mechanism of cold plasma treatment and its affectivity in acceleration of wound healing so this study will be focusing on the articles and researches that addresses its role in treating wounds, burns, bone graft and ulcerations and proved it.

The matter is divided into fourth states which are the solid, liquid, gases and also the plasma which is the fourth state of matter, plasma is an ionized gas and its classified into two types according to its temperature which are the hot or thermal plasma with a (4000-5000 k) and the cold or non-thermal temperature at (30-50°C) which usually help in generation of (negative and positive ions) in addition to nitrogen and oxygen free radicals (Martusevich et al., 2022) .

When considering the use of plasma in medical field the temperature of the plasma is very important because thermal or hot plasma can cause damage to the vital cells and organs that's why the non-thermal or cold plasma was the perfect choice since its temperature is close to room temperature.

The cold plasma mainly consists of metastables, ions, photons, electro-magnetic fields and electrons when non-thermal plasma reacts with the air, it will generate reactive nitrogen species (RONS) and reactive oxygen species (ROS) which helps in increasing the microcirculation of skin tissue, stimulate monocytes and keratinocytes, promotes and increasing the cell migration and finally stimulation of fibroblasts which play an important role in wound healing ((Friedman, 2020), (Braný, Dvorská, Halašová, & Škovierová, 2020).

The plasma production needs a constant energy source, many devices were made to generate cold plasma to be used in the biological and medical fields. These devices used different methods to generate the cold plasma like plasma needles, atmospheric plasma jets, plasma pencils and dielectric barrier discharges (D. Nguyen & Lee, 2016).

The cold plasma medicine is one of the modern medical fields which make use of the reactive species to solve many medical problems by targeting the biological tissues, it's very important option in cleaning, disinfection and healing of different types of wounds ( (Nasir, Lee, Yap, Thong, & Yap, 2016), (Hung et al., 2016)).

The inactivation process of bacteria is usually caused by biological and physical mechanisms. The cellular processes like DNA and cell membrane damage while the reactive species and free radicals are the physical mechanism. The cold atmospheric plasma was found to be very effective in wounds healing because of its ability to stimulate regeneration of tissues and inactivation of different types of bacteria (O'connor, Cahill, Daniels, Galvin, & Humphreys, 2014).

The cold plasma effects depends directly on the dose and duration of exposure, low doses of cold plasma result in very mild effects while high doses of cold plasma results in deadly effects (Eming, Krieg, & Davidson, 2007). The cold plasma has a deadly effect on the bacterial cells with very little effect and no damage on the living cells and plants

Using plasma in mild doses causes fibroblasts proliferation and play a very important role in wound healing especially chronic wounds and also helps in resistant bacteria, the cold plasma has the ability of increase the cell proliferation, make junctional protein changes, produce extracellular matrix protein and encourage the proliferation and migration of fibroblasts and keratinocytes, activate the gene expression of very specific gene like (type 1collagen, alpha smooth muscle actin and transforming growth factors ) that aids in wound healing, it also increase vascularization in the wound site and improve oxygen saturation and microcirculation ((Braný et al., 2020), (Haertel, Von Woedtke, Weltmann, & Lindequist, 2014)).

## 2. RESEARCH METHOD

This review study was done by using the reseachgate, PubMED and web of science databases and 187 researches and study were closely observed and studied and the the search words were (cold atmospheric plasma, wounds healing, plasma jet, medical applications of cold plasma and etc). Results showed thatthere are many studied that proved the efficacy of cold plasma in wound healing like its showed in the table (1).

**Table 1.** Plasma jet studies in wound healing process (Dubey et al., 2022)

| Delivery modality (jet, DBD) | Voltage waveform | Gas, flow, and pressure | Plasma information | Distance from applicator | Treatment time | Medical application and observation |
|---|---|---|---|---|---|---|
| Plasma jet | 6 kV, 1–2 μs pulse duration, 12 kHz PRF | He (8 L/min) | Average plasma power < 10 W | 3–4 cm | 1 min for every 5 min for 4 daily treatments | CAPP increased wound closure rate and may playa potential role in scar formation by inhibiting the TGF β1 signal pathway and reducing the levels of α-SMA and type I collagen, and may have clinical utility in the future |
| Jet | driving frequency 17 kHz, 1.33 $kV_{rms}$, 10.7 $mA_{rms}$ | He (11 slpm) | OH, $N_2$, He, O, NO, $N_2^+$ | | 1 min | Decreased wound area, increased wound contraction and re-epithelization, and increased protein level of smooth muscle actin. Increased inflammatory response with no changes in anti-inflammatory agents |
| Jet | 5 kV, 25 kHz, 4 slpm | He | OH, $N_2$ or NO, $N_2^+$, He, O, NO, and $N_2$ | 10 mm | 60 s, 3 times per day for 5 days | The plasma treated group exhibited accelerated pressure ulcer wound re-epithelialization, angiogenesis, and fibrosis than the untreated group with a shorter inflammation phase and significant acceleration of wound healing |
| Jet (microarray) | 20 kHz, 190–374 $V_{rms}$ | He (6 slm) | OH, $N_2$, $N_2^+$, He, O | 3 mm from tissue | 20–40 s/day | Seven days after wound formation, the wound area of the untreated control was 24 ± 5% of its initial value; an identical wound treated twice daily for 20 s was 9 ± 2% of its initial area |
| Jet | 20 kHz | Argon (5 slm) | OH, Ar1, O1 | 5 mm above the skin | 120 s | The ratio of the current wound area to the initial wound area of all the plasma-treated normal and diabetic groups decreased by up to 30% compared to untreated groups during healing. The overall results indicate that CAPP may increase the amount of free radicals involved in cell signaling, which may affect some pathways and accelerate healing of both normal and diabetic wounds |

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|---|---|---|---|---|---|---|
| Jet (microarray) | 20 kHz, 190–374 $V_{rms}$ | He (6 slm) | $N_2^+$, OH, $N_2$, He, $He_2$, O | 3 mm from sample | 20–40 s/day | Treating second-degree burns in animal rat models with CAPP reduced the wound area. Expression of interleukin (IL)-1α, -6 and -10 was verified to evaluate the healing effects. CAPP promoted re-epithelialization through collagen arrangement and the regulation of expression of inflammatory genes |
| Plasma jet | 1 $kV_{pp}$, 5 MHz | Helium, 1.75 L/min | PBS was administered topically to the wounds | 1 mm source-lesion distance | 2 min treatment daily using 16 sources on a mechanical support | Accelerated wound closure, enhanced vascularization, and reduced bacterial load due to CAPP exposure |

## 3. RESULTS AND DISCUSSIONS

In the reviewed and examined studies the cold plasma proved to be very effective in acute and chronic wound healing. Cold atmospheric plasma also has antiseptic effects that may promote wound healing and also be triggering the nitric oxide production (NO)that helps in cell migration and the gathering of endothelial cells in almost vessels structure to help in wound vascularization ((Duchesne, Banzet, Lataillade, Rousseau, & Frescaline, 2019), (Wang et al., 2020))

Cold plasma therapy can be modified according to the wound healing stages; helium plasma was found to be very effective in the healing process while the argon plasma was more effective in the coagulation stage (García-Alcantara et al., 2013).

Amini et al (Amini et al., 2020) found that the cold plasma therapy helps in modification of growth factors like (TNF-α, IL-8, TGF-β, IL-1, INF-γ) and cytokines to improve the healing process by fast initiation and also proliferation stage. And also found that cold plasma has the ability to generate reactive nitrogen species (RNS) and reactive oxygen species (ROS) which help in the production of proangiogenic factors and improve wound healing process ((Xu et al., 2015), (Martines et al., 2020)**)**

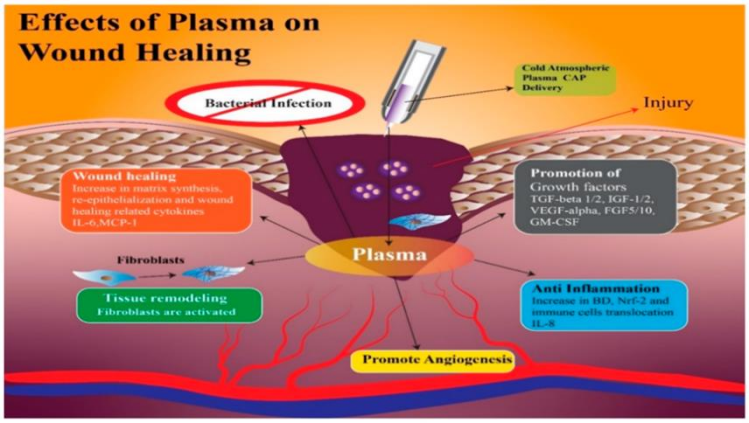


**Figure 1.** The application of CAP on the wound healing (Bolgeo et al., 2023).

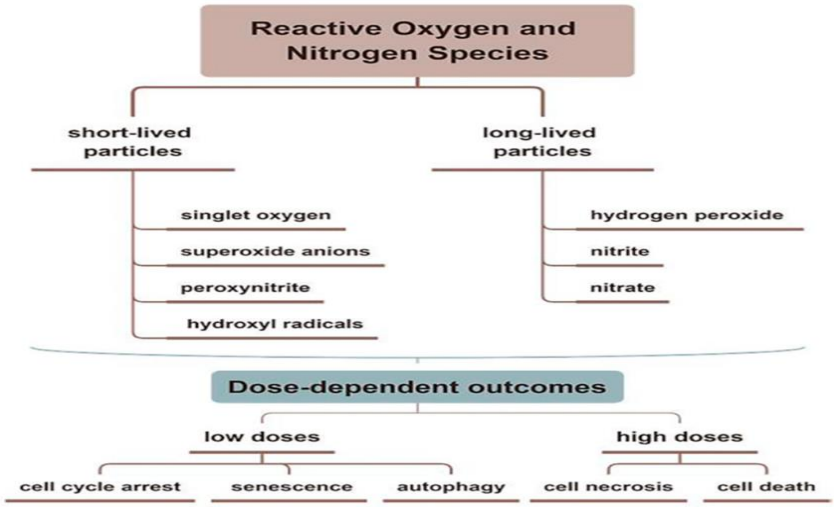


**Figure 2.** Reactive oxygen species (ROS) and reactive nitrogen species of (CAP) which help in cell migration and increase proliferation to help in wound healing (Bolgeo et al., 2023).

Gao et al (Gao et al., 2019) demonstrated the cold plasma therapy effects on traumatic wounds, the first patient used in effective wound treatment and suffer from secondary eczema and exudates and he was treated with cold plasma therapy for (20 mins every day and results were very promising after 3 session, cold plasma was found to be very effective in exudates prevention and wound healing acceleration while the second patient was treated unsuccessfully with different antibiotic and he showed complete wound healing after being treated with cold plasma therapy for 3sessions Heinlin et al (Heinlin et al., 2013) studied the effect of argon cold plasma on wound healing in skin grafts donors in the upper legs, he included 40 patients in this study and he divided the study samples into two groups one treated with placebo argon gas and the other with argon cold plasma for 2mins every day and in the second day the plasma treated wounds were found to show great improvement in re-epithelialization, fibrin layers reduction and increase in blood flow compared to the placebo group

Nguyen et al (T. X. Nguyen, Nguyen, Ho-Man, Bui, & Phan, 2022) studied the effect of cold plasma on different wounds like burns, soft tissue wounds, shingles, atopic dermatitis and pressure ulcers and found that 70% of the study sample patients showed complete wound healing and re-epithelialization after 14 days of CAP treatment and the author mentioned these wounds usually take so much longer in treating wounds.

Many others studied the cold plasma therapy was found to has a great role in many medical and biomedical application that have benefits in humans lives like inhibition of bacterial growth and improve chronic wound healing and diabetic foot ((Becker et al., 2017), (Li et al., 2023),(Masur, 2023),(Schleusser et al., 2022),(Bagheri et al., 2023),(Yoo, Kang, Baek, & Hwang, 2023)).

## 4. CONCLUSION

One of the main problems in medicine is the recurrent infection and delayed wounds healing which might affect the general patients health (Everett & Mathioudakis, 2018), in the recent years cold atmospheric plasma was started to be used in medicine especially cold atmospheric plasma was found to be very effective as anti-inflammatory, anti-tumor and antimicrobial effects in medicine ((Cheng et al., 2018),(García-Alcantara et al., 2013),(Garner & Mehlhorn, 2021)) and cause no damage to the living tissues and cells because of its low temperature besides it has the ability to improve and accelerate wounds healing through specific mechanisms which help in cell migration and proliferation in addition to increase the vascularization at the wound site and help in gathering the fibroblasts and keratinocytes and the production of reactive nitrogen and oxygen species (RNS,ROS). The efficiency of cold plasma therapy depends on device related factors which are the treatment time, the device design, the gas type and flow rate, plasma frequency and intensity, the distance between the plasma source and the sample while the second is wound related factors like exudates, extracellular matrix

and wound type. In the future more benefits of cold atmospheric plasma will be studied and discussed.

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