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REVIEW

Cold atmospheric plasma (CAP) in wound healing: harnessing a dual-edged sword

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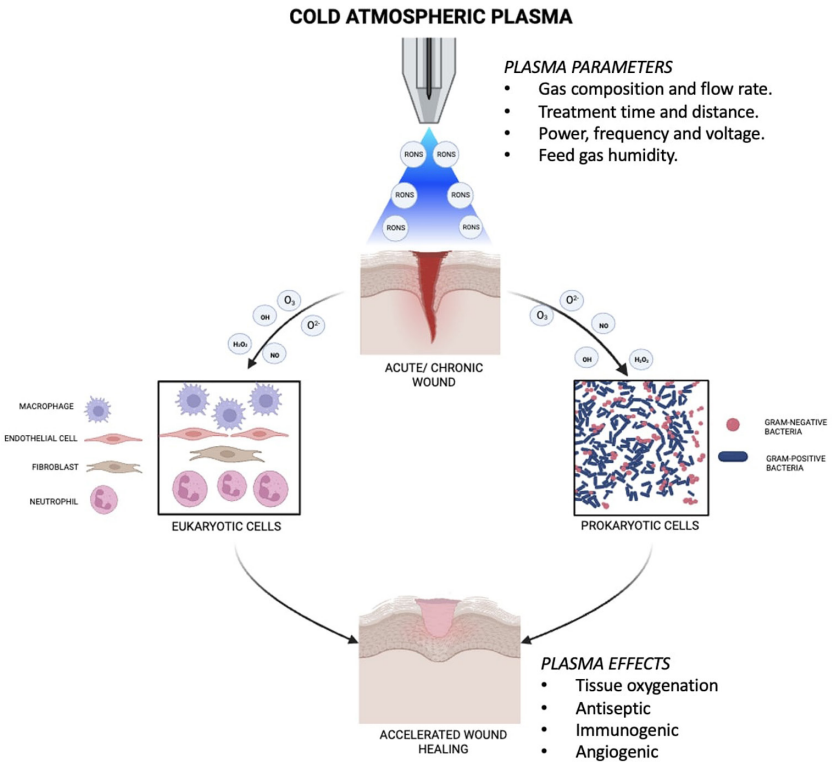
Graphical abstract



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Abstract

Chronic wounds take longer to heal and, if left untreated, can result in severe repercussions such as sepsis, gangrene, and amputation. The current treatment procedures followed are wound cleaning and debridement, specialized dressings, antibiotics and antiseptics, hyperbaric oxygen therapy, and vacuum-assisted wound closure. Some of the limitations of these treatment options are multidrug resistance and tissue toxicity. Cold plasma is an emerging technology that has opened a new frontier in biomedical applications and is found to have great utility in wound healing. Cold plasma comprises reactive oxygen and nitrogen species (RONS) that can be targeted against bacterial inactivation and improve wound healing. The amount of RONS produced can be controlled by several parameters such as gas composition, flow rate, power, frequency, voltage, distance, and exposure time. The reactive species causes damage to the cell membrane as well as the intracellular components which ultimately lead to bacterial cell death. It can also accelerate wound healing by activating neutrophils, macrophages, endothelial cells, keratinocytes, and fibroblasts. These help in maintaining tissue oxygenation, initiating angiogenesis, collagen synthesis which aids in rapid wound closure. In this review, we summarize the various characteristics of cold plasma that can be optimized to produce an effective antimicrobial effect. The different mechanisms of bacterial inactivation and the stimulation of wound healing processes by the reactive species are discussed. Furthermore, numerous pieces of evidence from *in vitro* and *in vivo* experiments and clinical trials that prove that cold plasma is an effective approach are presented.

Keywords: cold atmospheric plasma; reactive oxygen species; chronic wounds; antimicrobial effects

Introduction

Matter exists in four states: solid, liquid, gas, and plasma. Plasma, although uncommon on earth, makes up 99% of the visible universe. Plasma is partially ionized gas with charged and uncharged entities. Energy processes in the form of heat or electromagnetic radiation in combination with gases such as argon, helium generate plasma, consisting of charged ions and are an effective conductor of electricity (Hoffmann *et al.* 2013, Niedzwiedz *et al.* 2019, Tabares & Junkar 2021). There are two types of plasma: thermal and nonthermal (cold atmospheric plasma (CAP)), with thermal plasma having a temperature range of 10^7–10^9°C (Wesson 1978, Tabares & Junkar 2021). Nonthermal plasmas, also known as cold atmospheric plasmas, have temperatures similar to room temperature. Cold plasma technology uses low-pressure ionized gases for challenges in health care, materials science, and environmental remediation (Lin *et al.* 2022). Various methods have been developed to produce CAP, such as dielectric barrier discharge (DBD), atmospheric pressure plasma jet (APPJ), plasma needle, and plasma pencil (Hoffmann *et al.* 2013). The utilization of plasma is capable of generating reactive oxygen and nitrogen species (RONS), thereby enabling the destruction of detrimental bacteria, viruses, and fungi, thus facilitating the disinfection of surfaces, equipment, and human epidermis (Brany *et al.* 2020, Tabares & Junkar 2021). In addition, the cold plasma technology is currently under exploration for the treatment of wound healing, and tissue regeneration (Hoffmann *et al.* 2013, Hu & Zhang 2020, Faramarzi *et al.* 2021). As a nonthermal method, cold plasma technology presents the advantage of not causing damage to heat-sensitive tissues, making it a potential wound healing solution. CAP is identified as an effective and reliable treatment modality against various dermatological conditions and is also currently under investigation for the treatment of skin tumors (Bernhardt *et al.* 2019). Even though CAP is a novel therapeutic strategy, CAP was also found to be safe to use for treatment as using this does not show any harmful effects (Friedman 2020). In the following review, we discuss the tunable physical properties of CAP, current review of literature on microbial inactivation, and tissue oxygenation governing wound healing mechanisms. We further summarize the clinical studies and case reports on efficacy of CAP in the treatment of acute and chronic wounds.

Characteristics of CAP

In order to understand the behavior of the CAP, it is important to consider the different parameters that decides the quality of it. Since the CAP devices are used in clinical practices, it is necessary to reduce side effects and should be designed to avoid toxicity in the healthy cells. This is achieved by adjusting the different parameters that are required for effective and optimal dosage (Fig. 1). The efficiency and efficacy of CAP depend on several tunable parameters, including the gas composition, flow rate, power input, and electrode configuration.

Gas composition and flow rate

Neutral gases like helium, argon, heliox (a combination of helium and oxygen), and nitrogen can be used to

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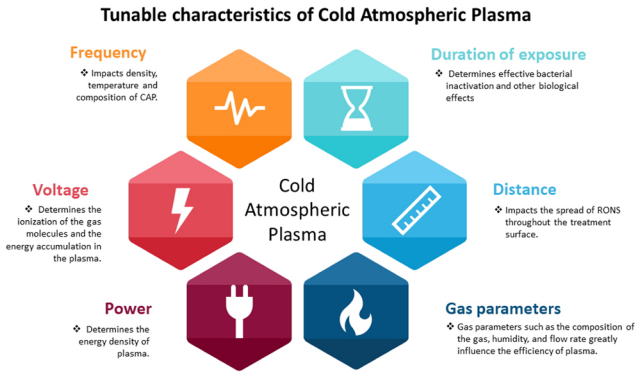

Figure 1

Tunable characteristics of CAP. The schematic representation of different tunable parameters that determine the efficacy, quality and behavior of CAP. Adjusting these parameters can produce different types of RONS as well as its dissemination over the treated surface. CAP, cold atmospheric plasma; RONS, reactive oxygen and nitrogen species.

generate CAP, with nitrogen being commonly used due to its antibacterial effects (Hoffmann *et al.* 2013). The gas flow rate has an impact on the behavior of the plasma and a flow rate of 2.2 L/min for argon and 2 L/min for helium have been found effective for treating chronic ulcers. Higher flow rates result in a more homogeneous plasma and can eliminate any by-products produced during the plasma generation process (Isbary *et al.* 2012, Mirpour *et al.* 2020).

Treatment time and distance

The duration of plasma exposure and distance to treatment surface are important parameters to achieve efficacy and off target toxicity. Effective CAP treatment requires consideration of cancer/infection type, tissue density/thickness, plasma source power/frequency, and gas composition (Brany *et al.* 2020). A 2-min and 5-min exposure of argon-supplied CAP effectively treats chronic wounds (Isbary *et al.* 2010, 2012). Varying effects of plasma are influenced by distance, with significant differences observed when reducing distance from 15 mm to 5 mm between tissue and nozzle (Vasile Nastuta *et al.* 2013, Brany *et al.* 2020).

Power, frequency, and voltage

The power input is a significant factor in CAP as it determines the plasma's energy density. It is governed by voltage, frequency, and electrode design. Increased power input leads to higher plasma densities and more reactive species, resulting in greater efficacy (Duan *et al.* 2007). Conversely, decreasing power input can lower the concentration of reactive species (Mai-Prochnow *et al.* 2021). However, excessive power input can cause thermal effects that may be harmful in biological applications. Hence, it is crucial to determine the best power input for every use, ensuring the plasma's effectiveness and safety. Additionally, frequency plays a significant role in CAP, representing the electrical signal frequency used to produce the plasma. The properties of plasma can be affected by the frequency of the electrical signal, including temperature, density, and composition (Tabares & Junkar 2021). High-frequency signals create stable and uniform CAP, while pulse duration affects plasma–surface interaction. Low-frequency signals produce energetic plasma but can cause damage due to longer interaction times. Voltage is a crucial factor in determining CAP formation. It represents the electric field strength applied to the gas, which determines the ionization of gas molecules and energy accumulation in the plasma. Voltage control can result in different plasma conditions, such as varying reactive species density, temperature, and discharge power. Voltage can be regulated by adjusting the frequency and amplitude, ranging from several hundred volts to a few kilovolts. Higher voltage leads to a higher density of reactive species and increased plasma temperature, but excessive voltage can damage equipment and cause undesired reactions such as arcing which can damage the equipment (Kim *et al.* 2022). Cold plasma produced by applying a voltage of 5 kV and frequency of 25 kHz to helium gas was shown to accelerate the rate of wound healing of pressure ulcers in animal models (Chatraie *et al.* 2018).

Feed gas humidity

The stability of plasma is influenced by feed gas humidity. Humidity plays a crucial role in plasma-related chemical reactions involving dissociation of water molecules. Humidity is introduced through impurities or diffusion into the effluent in humid environments. Humidity changes not only affect plasma jets but also generate free radicals such as OH^- and H_2O_2, causing oxidative stress in biological samples (Kirkpatrick *et al.* 2008, Winter *et al.* 2013).

Although several of these parameters determine the quality of plasma output, studies predominantly rely on tuning feed gas composition, treatment height, and duration of exposure to determine the optimal clinical response.

Action of CAP on microbial inactivation

A wound is defined as a disruption in the skin or other tissues resulting from external injuries or surgical incisions (Kujath & Michelsen 2008). These injuries are colonized by a plethora of microorganisms, some of which have the potential to be pathogenic, generate virulence factors, and elicit multidrug resistance. This enhances the likelihood of wound infections and protracts the wound healing process. Microbes,

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primarily bacteria, that infiltrate wounds originate from three sources, namely, the environment, the surrounding skin, and endogenous sources (Bowler *et al.* 2001). Commonly found microorganisms in wounds include aerobic bacteria, anaerobic bacteria, and fungi. Gram-positive and gram-negative bacterial species of various sorts have been detected within the wounds (Puca *et al.* 2021). The resultant infection can arise from a specific bacterial strain (monomicrobial) or through the actions of multiple bacterial strains (polymicrobial) (Anju *et al.* 2022).

In the context of chronic wound microbiology and those who have had hospital acquired infections, gram-negative bacterial strains appear to be more prevalent than their gram-positive counterparts. Among the gram-positive bacteria that are predominantly encountered in wounds are *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis*, *Corynebacterium* spp., and *Staphylococcus epidermidis*. Conversely, the predominant gram-negative bacteria found in wounds are *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Proteus mirabilis*, and *Acinetobacter baumannii/haemolyticus* (Dowd *et al.* 2008, Banu *et al.* 2015, Bessa *et al.* 2015, Sadeghpour Heravi *et al.* 2019, Chai *et al.* 2021, Puca *et al.* 2021, Hassan *et al.* 2022). Additionally, fungal species, such as *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Trichophyton rubrum*, *Aspergillus niger*, and *Aspergillus fumigatus*, have also been identified in wound samples (Kandregula *et al.* 2022).

CAP is a useful tool for medical and biological purposes, as it can reduce bacterial load in wounds and initiate healing. The effectiveness of CAP treatment can be affected by intrinsic properties of microbes such as their morphology and physiology. Gram-negative bacteria are more sensitive to cold plasma treatment than gram-positive bacteria due to the thinness of their peptidoglycan layer (Laroussi & Leipold 2004*a*, Mai-Prochnow *et al.* 2016). A prolonged course of treatment with CAP is necessary for effective eradication of bacterial spores when compared to vegetative bacteria due to the former's impermeable multilayered structure, which impedes the penetration of plasma and the targeting of cellular components (Liao *et al.* 2019). The fungal cell wall, composed of chitin, exhibits higher resistance to CAP treatment than bacteria, primarily due to chitin's heightened rigidity in comparison to peptidoglycan (Mravlje *et al.* 2021). Furthermore, the physiological stage of bacteria is a crucial factor that impacts the efficacy of CAP, as demonstrated by the fact that *Escherichia coli* was more effectively reduced when exposed to CAP during the 12-h growth phase compared to the 24-h and 48-h phases for the same exposure duration (Deng *et al.* 2007). Bacterial inactivation can be achieved through direct or indirect treatment with plasma. Direct treatment involves the use of plasma components such as heat, UV radiation, RONS, and charged particles. Studies show that UV radiation and heat are not significant in bacterial inactivation, while charged particles are effective in inactivating gram-negative bacteria due to their unique outer membrane (Breijyeh *et al.* 2020). RONS produced by CAP are responsible for bacterial inactivation (Laroussi & Leipold 2004*b*, Das 2022). Proteins are fundamental constituents of all living organisms and are paramount in upholding structural and functional integrity of cells. Multiple inquiries have demonstrated that CAP can initiate oxidation of amino acid residues in proteins, resulting in protein fragmentation and denaturation. This process can disturb the operation of critical proteins involved in bacterial metabolism, DNA replication, and transcription, ultimately culminating in cell death (Afshari & Hosseini 2013, Niedzwiedz *et al.* 2019). Correspondingly, CAP can also interact with lipids, which are crucial components of bacterial membranes. CAP can trigger lipid peroxidation, giving rise to the creation of reactive oxygen species and lipid hydroperoxides. This can lead to damage of the membrane and changes in the membrane's permeability, resulting in cell death (Lv & Cheng 2022). These compounds constitute fundamental constituents of the bacterial cell wall and serve a pivotal function in preserving the cell wall's structural robustness. The impact of CAP on polysaccharides is manifested through the oxidation of sugar residues, ultimately resulting in cell wall impairment and bacterial cell lysis. Figure 2 depicts the different mechanisms involved in bacterial inactivation.

CAP causes the rupture of the bacterial cell membrane in the following ways:

Lipid peroxidation

Lipids are a crucial constituent of the plasma membrane. The majority of the lipids responsible for the formation of the bacterial plasma membrane comprise polyunsaturated fatty acids (PUFA). The role of PUFA involves imparting membrane fluidity (Harayama & Shimizu 2020). Notably, PUFA is particularly susceptible to the adverse effects of ROS, particularly OH radicals, due to its location in close proximity to the cell surface. The process of lipid peroxidation entails the attack of free radicals on lipids containing carbon–carbon double bonds, such as PUFA. This reaction involves the extraction of H atom from PUFA by ROS, resulting in the formation of a fatty acid radical (L•). The radical is then oxidized, generating lipid hydroperoxide (LOOH) (Brelles-Marino *et al.* 2006, Joshi *et al.* 2011). The products of these reactions, including hydroperoxides and other lipid peroxides like malondialdehyde, can compromise the efficacy of membrane lipids (Hosseinzadeh Colagar 2013, Wang *et al.* 2018). *E. coli* treated with FE-DBD produced ROS inducing lipid peroxidation, leading to irreversible DNA and protein damage and covalent adduct formation. In gram-negative bacteria, ROS acts on membrane lipids via peroxidation, while in gram-positive bacteria, ROS

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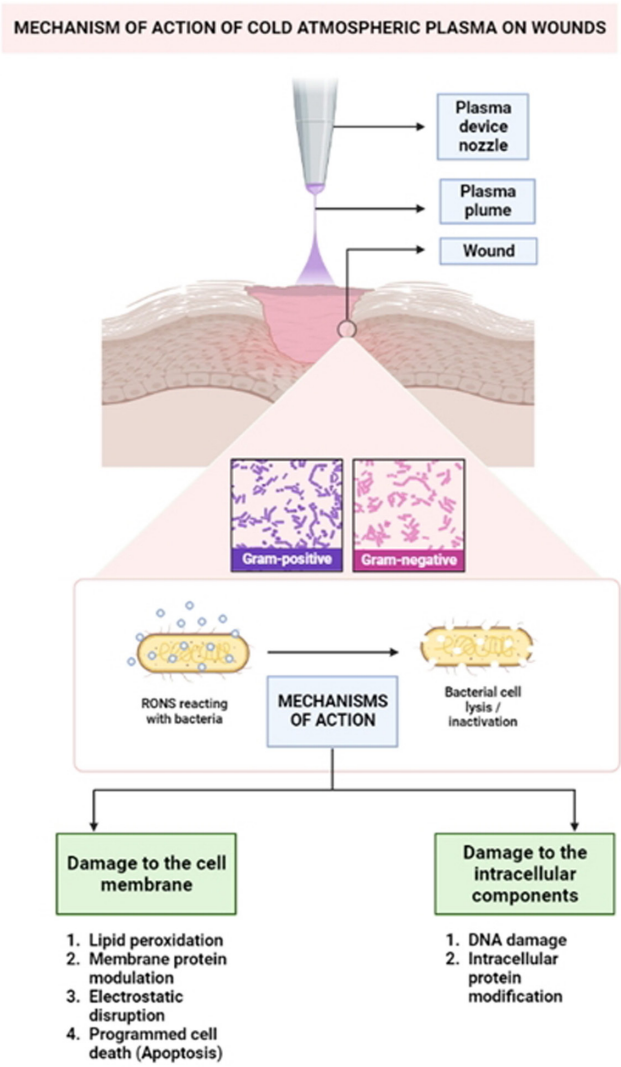

Figure 2

The mechanism of action of CAP for bacterial sterilization. Schematic representation of CAP disinfecting the wounds that are colonized either by gram-positive and gram-negative bacteria which delays wound healing. The RONS produced by the CAP enters the bacterial cell through the disruption of the cell membrane and causes DNA damage and protein modifications leading to bacterial inactivation. RONS, reactive oxygen and nitrogen species; CAP, cold atmospheric plasma.

causes oxidative damage to intracellular components (Han *et al.* 2016).

Protein modulation

Protein modification has emerged as a significant process in the domain of protein chemistry owing to its potential to augment the protein quality and its functional attributes. CAP has been demonstrated to elicit protein modifications, which, in turn, facilitates the production of bactericidal effects. The deployment of SDS-PAGE and 2D-PAGE techniques have revealed that OH radicals can trigger chemical modifications and degradation of membrane proteins (Digel *et al.* 2005). However, no alterations were discerned in the DNA or cytoplasmic protein. Other studies have also furnished evidence by demonstrating changes in electrophoretic patterns resulting from the degradation of proteins triggered by OH radicals (Venezia *et al.* 2008, Hosseinzadeh Colagar *et al.* 2013). Moreover, proteins are susceptible to oxidation by atomic oxygen or metastable oxygen molecules (Laroussi 2008). Therefore, it has been empirically confirmed that plasma treatment induces alterations in the 3D structure of proteins (Xinyu Liao 2017).

Electrostatic disruption

The utilization of plasma membrane disruption has proven to be an effective sterilization strategy for bacteria (Mason *et al.* 2006). The most efficient process involves the accumulation of charged particles that are generated by the cold CAP on the bacteria's surface. This results in electrostatic stress that can overcome the cell wall's tensile strength, ultimately causing the cell membrane to rupture and leading to bacterial cell death. This mechanism of action is predominantly observed in gram-negative bacteria, which possess an outer membrane and a thin layer of peptidoglycan that can be easily disrupted, causing the cell to leak (Xinyu Liao 2017).

Programmed cell death

ROS has played a significant role in multiple cell signaling pathways and has been observed to induce oxidative stress and apoptosis, a specific form of programmed cell death. Furthermore, plasma, a crucial initiator of redox mechanisms, has been identified to stimulate apoptosis in bacterial cells. Research has demonstrated that bacterial programmed cell death occurs within a mere 15 s of exposure time when utilizing high- and low-voltage DBD (Lunov *et al.* 2015). Additionally, the accumulation of intracellular ROS has been found to prompt apoptosis in bacteria (Xinyu Liao 2017). In another study, it was shown that CAP triggers physical destruction of bacteria or programmed cell death with the characteristics of apoptosis in both gram-negative and gram-positive bacteria (Lunov *et al.* 2016).

Cold plasma causes oxidative damage to the bacterial cells by the following mechanisms:

Deoxyribonucleic acid

The generation of hydroperoxide groups in the DNA structure through the action of reactive oxygen and nitrogen species (RONS) is responsible for the oxidation of DNA (Valverde *et al.* 2018). The resulting formation of intramolecular crosslinks with nucleic acids and proteins

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can lead to irreparable damage (Niedzwiedz *et al.* 2019). It should be noted that CAP not only produces RONS but also generates lower dosages of UV radiation compared to traditional UV-light systems employed for sterilization purposes (Rastogi *et al.* 2010, Golda *et al.* 2020). This type of UV radiation generated by CAP has been shown to interact with the cell wall and cause DNA damage leading to cell death (Niedzwiedz *et al.* 2019, Das 2022).

Intracellular proteins

Plasma possesses the capacity to induce degradation of cellular proteins (Niedzwiedz *et al.* 2019, Anne Mai-Prochnow *et al.* 2022). The reactive species instigate the cleavage of hydrogen, sulfide, and peptide bonds, leading to alterations in the primary, secondary, and tertiary structures of proteins. The anomalous configuration of the protein brings about a decline in enzymatic activity within the cell (Niedzwiedz *et al.* 2019). Through the of gel electrophoresis, it was discovered that there was a decrease in the concentration of protein levels as the exposure time to CAP increased. This phenomenon resulted from the peptide bond breakage, subsequently exposing the amino acids present (Hosseinzadeh Colagar *et al.* 2013). In contrast, a study has indicated that bacteria express proteins excessively when they are exposed to CAP, with most of these proteins associated with carbohydrate and nucleotide metabolic pathways. The overexpression of these proteins is carried out as a crucial part of the bacterial defense mechanism against CAP (Attri *et al.* 2018).

Biofilm

A biofilm is a membranous tissue formed over the surface of chronic wounds by a structured community microorganisms encased in an exopolysaccharide substance (Wei *et al.* 2019). Biofilm is polymicrobial as it consists of bacteria, fungi and viruses and other biotic components such as proteins and extracellular DNA. Biofilms supports bacterial growth by providing protection even in hostile conditions (Costerton *et al.* 1978, Fuxman Bass *et al.* 2010, Clinton & Carter 2015). The bacterial biofilms on chronic wounds makes them blatantly resistant to frontline antibiotics when compared to the free-living microbes due to the acidic and hypoxic microenvironment (James *et al.* 2008, Chiang *et al.* 2013).

Studies have shown the efficacy of CAP on treatment of bacterial biofilms by effectively disrupting biofilms structural integrity and targeting microorganisms within. CAP was found to inactivate the monospecies biofilms formed by *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA) (Brun *et al.* 2018). CAP was also found to inactivate the biofilms formed by *E. faecalis* (Theinkom *et al.* 2019). A study by Matthes *et al.* showed that six-fold repeated application of CAP on *Staphylococcus aureus* present on the biofilm did not cause resistance or habituation against the plasma (Matthes *et al.* 2014). Recent studies have shown that synergistic effects of CAP and antibiotics can significantly enhance the efficacy of biofilm eradication. CAP treatment increases the permeability of biofilm, thereby enhancing the penetration of antibiotics (Brun *et al.* 2018). Furthermore, the synergy between cold plasma and antibiotics can not only eradicate the biofilm but also prevent the emergence of antibiotic-resistant strains, making it a potential solution against antibiotic tolerance (Maybin 2023). The proliferation of microbial infections associated with medical devices has recently garnered public health attention in light of the emergence of diverse transplantable devices. Presently, the coating of these devices with antibiotics serves as a viable measure to deter the formation of biofilm. However, a recent investigation has demonstrated that a synergistic amalgamation of antibiotics and CAP not only reduced the bacterial load, but also impeded the formation of biofilm, proving to be a more effective strategy (Los *et al.* 2019).

Effects of CAP on wound healing

The wound healing process occurs in four stages: homeostasis, inflammatory phase, proliferation phase, and remodeling phase. In chronic wounds, these stages are altered and often remain in the inflammatory phase (Frykberg & Banks 2015, Marches *et al.* 2022). RONS are produced in the injury site by cells involved in healing, particularly neutrophils and macrophages. Neutrophils and macrophages generate superoxide and H_2O_2 through the NADPH oxidase complex (Mittal *et al.* 2014). NO is also produced by macrophages, which reacts with oxygen to form peroxynitrite (Prolo *et al.* 2014). Fibroblasts produce ROS through mitochondria, which drives the epigenetic changes that helps in differentiation of these cells (Shrishrimal *et al.* 2019). Endothelial cells produce ROS through the NADPH oxidases, with NOX-2 and NOX-4 being predominantly induced during pathological stimuli (Winterbourn *et al.* 2016). The principal RONS that exert a significant influence on the healing process are hydrogen peroxide (H_2O_2) and nitric oxide (NO). The levels of ROS prove to be detrimental to the aforementioned process. While lower concentrations of ROS facilitate the phase transition, increased concentrations lead to oxidative stress and programmed cell death via apoptosis, necroptosis, and immunogenic cell death (Forman & Torres 2001, Fan *et al.* 2014, Janda *et al.* 2016, Privat-Maldonado *et al.* 2019).

During wound healing, thrombin and vascular constriction prevent excessive bleeding and stimulate platelet derived growth factors and selective entry of the inflammatory cells into the wound area (Zhu *et al.* 2017, Bekeschus *et al.* 2021). RONS play a key role in platelet recruitment and activation (Qiao *et al.* 2018, Masselli *et al.* 2020). Hypoxia is an initiator of the healing process,

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leading to increased activity of ROS and regulation of tissue oxygen homeostasis. This hypoxic condition leads to the transcription of HIF genes leading to oxygenation and boosting the activity of ROS, ultimately regulating the tissue oxygen homeostasis in the wound (Kimmel *et al.* 2016). ROS will also initiate nuclear factor erythroid 2-related factor (Nrf2; redox sensitive transcription factor) signaling pathway, which maintains the redox homeostasis. This will also activate several other pathways to protect the cells against oxidative stress or damage (Privat-Maldonado *et al.* 2019, Boeckmann 2020). RONS induces chemokines and cytokines for neutrophil and macrophage recruitment during inflammation, with concentration dependent effects (Kimmel *et al.* 2016, Boeckmann 2020). Inflammation phase sees gradual increase in H_2O_2 levels, which attract phagocytes to injured region. H_2O_2 triggers expression of mRNA proteins that act as chemo attractants (Zhu *et al.* 2017, Privat-Maldonado *et al.* 2019). H_2O_2 also upregulates inflammation related genes for cytokine secretion and growth factor production in various cell types of tissues. Nitric oxide is synthesized by the macrophages and neutrophils via the inducible nitric oxide synthetase (iNOS) in the early stages of inflammation. The cytokines IL-1, TNF-α, and γ-interferon are responsible for the induction of iNOS activity (Witte & Barbul 2002). Furthermore, the Nrf2 signaling pathway contributes to the inflammatory events by promoting cytokine secretion and early infiltration of inflammatory cells (Privat-Maldonado *et al.* 2019).

During wound healing, various processes occur including reepithelialization, angiogenesis, and collagen synthesis (Bekeschus *et al.* 2021). H_2O_2 promotes keratinocyte migration and fibroblasts secrete CTGF and Cyr61 to activate keratinocytes. Release of heat shock proteins and fibroblast growth factor enhances neuronal survival and angiogenesis (Zhu *et al.* 2017, Boeckmann 2020). Angiogenesis requires growth factors, cytokines, and RONS, involving endothelial cell proliferation and recruitment of perivascular cells (Arndt *et al.* 2013). Granulation tissue formations occur due to fibroblast activity. Collagen synthesis depends on NO synthesis and iNOS activity impacts collagen production. NO also stimulates keratinocyte proliferation and reepithelialization through vascular endothelial growth factor (VEGF) (Witte & Barbul 2002, Gonzalez *et al.* 2016).

The phase of wound closure, identified by the cessation of remodeling, is a process that has a duration of approximately 2 weeks to 2 years. The involvement of collagen and various cell types facilitate the reorganization of the extracellular matrix to promote wound area closure (Bekeschus *et al.* 2021). H_2O_2 serves to upregulate the expression of TGF-1, which subsequently induces a stimulatory response to NOX-2 and NOX-4. These molecules play a crucial role in the differentiation of dermal fibroblasts and the subsequent deposition of collagen (Zhu *et al.* 2017).

Since, healing of chronic and acute wounds is mediated by physiological RONS, makes CAP an excellent tool not only in eradicating microbial infection but also activating tissues for wound healing in chronic wounds by delivering exogenous RONS (Privat-Maldonado *et al.* 2019, Boeckmann 2020). Several studies have reported the penetration depth of CAP derived ROS in human skin models ranging from 10 µm to 400 µm (Privat-Maldonado *et al.* 2019). CAP is known to generate various RONS, including superoxide ($O^{2\bullet-}$), singlet delta oxygen ($_1O^2$), atomic oxygen (O), hydroxyl radical (•OH), hydrogen peroxide (H_2O_2), nitrogen dioxide radical ($\bullet NO_2$), peroxynitrite (ONOO−), and nitric oxide (•NO) (7). The deposition of these oxidants and free radicals activates several tissue types via molecular mechanisms similar to physiological conditions. During CAP treatment, Keap1 stress sensor recognizes ROS and activates downstream Nrf2 signaling. Nrf2 is released from Keap1 by oxidation reactions and upregulates antioxidant responsive elements to detoxify ROS (Ma 2013). Nrf2 signaling regulates immune cells, angiogenesis, growth factors, and cellular viability during inflammation and promotes wound healing (Privat-Maldonado *et al.* 2019, Boeckmann 2020) (Fig. 3).

Cold plasma treatment leads to an increase in various pro-inflammatory factors such as IL-6, IL-8, MCP-1 of IL-8, CXCL-1, TGF-β1, and TGF-β2, expressed post injury in fibroblasts and keratinocytes (Arndt *et al.* 2013, Boeckmann 2020). Keratinocytes show an increase in b1-integrin expression and a reduction in E-cadherin and EGFR expression after 30 s of treatment. The process of establishing homeostasis promotes cell proliferation and differentiation.

Plasma treatment enhances neovasculature by modulating endothelial nitric oxide synthase signaling (Xiong 2018). Various angiogenesis related molecules are expressed including EGF, EG-VEGF, endothelin 1, FGF-2, IL-8, urokinase-type plasminogen activator, angiogenin, Col18A1, MCP-1, MMP-9, TIMP-1, and amphiregulin in fibroblasts, endothelial cells, and keratinocytes. Pro-angiogenic factors are also modulated through autocrine and paracrine signaling (Xiong 2018, Boeckmann 2020).

The utilization of Cold plasma treatment has a positive impact on MCP-1, an essential chemokine that stimulates the expression of collagen type 1 in fibroblasts. This is achieved through the endogenous upregulation of TGF-β (Arndt *et al.* 2013). An increase in the expressions of α-SMA, TGF-β-1, TGF-β-2, MCP-1, IL-6, and collagen type 1 in fibroblasts was observed post exposure to CAP. This observation suggests that the cascade of expressions required for the healing process is promoted by cold plasma technology (Arndt *et al.* 2013). Studies have shown that exposure to cold plasma increases the level of ROS in cells involved in wound healing. This, in turn, increases vascular flow, thereby increasing oxygen content in the tissue.

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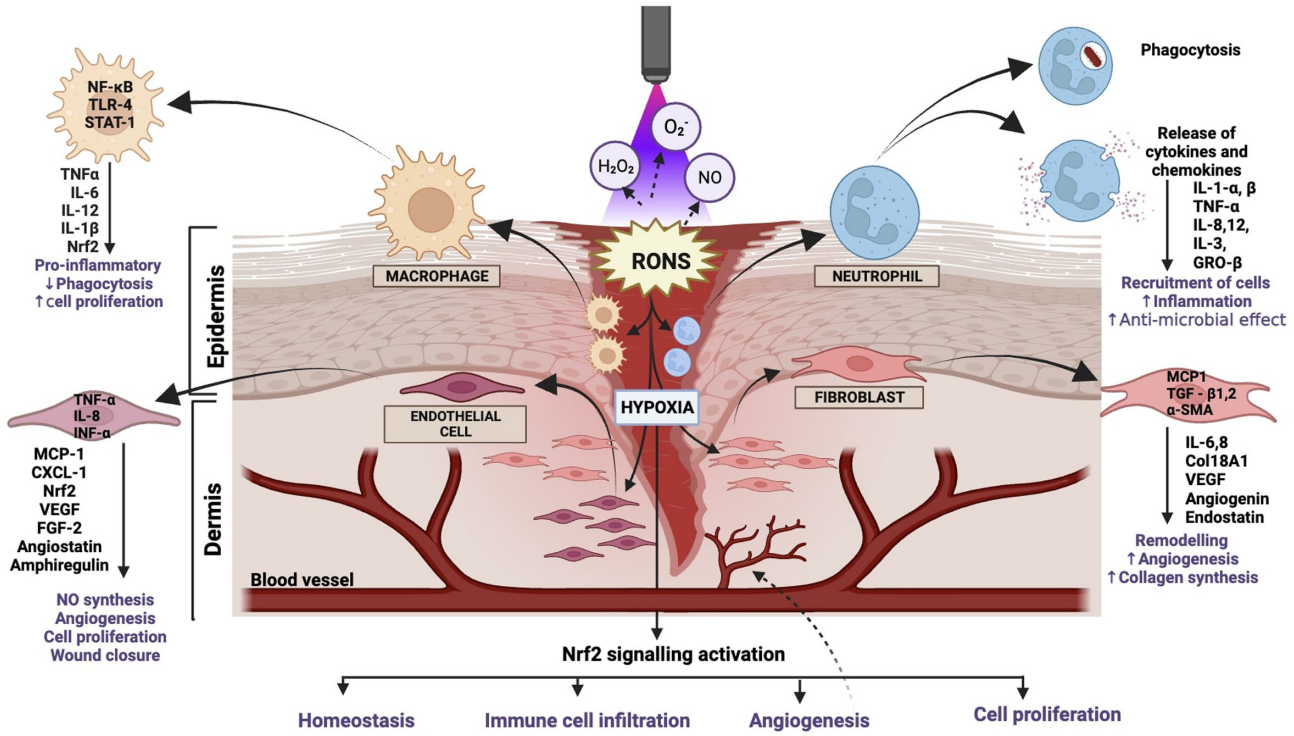

Figure 3

The modality of function of CAP in wound healing. Schematic representation of various molecular mechanisms that are activated by the RONS in respective cell types. RONS activates the neutrophils for phagocytosis and the release of cytokines and chemokines leading to the recruitment of inflammatory cells to the wound region which results in increased inflammation and increased antimicrobial activity. The activation of macrophages is instigated by the process of activation of different molecules such as NF-κB, TLR-4, and STAT-1. These activated macrophages induce pro-inflammatory effects, increase cell proliferation and reduced phagocytosis. RONS induces the activation of fibroblast by MCP1, TGF-β1 and 2, α-SMA, which regulates collagen synthesis, angiogenesis, and remodeing of the wound. RONS also activates cytokines such as TNF-α, IL-8, and INF-α in the endothelial cells and regulate nitric oxide synthesis, angiogenesis, cell proliferation, and wound closure. Cold plasma produced RONS in the presence of hypoxia activates the Nrf2 signaling which is responsible for regulating various processes such as angiogenesis, immune cell infiltration and cell proliferation. CAP, cold atmospheric plasma; RONS, reactive oxygen and nitrogen species; NO, nitric oxide; H_2O_2, hydrogen peroxide; $O_2^{\bullet-}$, superoxide.

Hyperspectral imaging (HSI) is an emerging imaging technique that combines spectroscopy and digital image analysis (Daeschlein *et al.* 2018, Bekeschus *et al.* 2020). This noninvasive technique is used to visualize the microcirculation and oxygen concentration post plasma treatment in the wound and surrounding region (Daeschlein *et al.* 2018, Borchardt *et al.* 2022). The medical application of HSI records a spectral range between 500 nm and 1000 nm and provides three-dimensional data on oxygen saturation in superficial and deeper layers. In addition, it can be employed to detect hemoglobin and water content in deeper layers in real time (Bekeschus *et al.* 2020, Borchardt *et al.* 2022). Recent study using this technology observed that CAP treatment induced accelerated wound healing by enhancing oxygenation and perfusion in tissue when using HSI in their studies. They also noted higher hemoglobin levels immediately after treatment and reduction in edema in the wounded tissue site (Bekeschus *et al.* 2020). Borchardt *et al.* monitored the microcirculatory response of the intact human skin post plasma treatment using noninvasive optical methods and found that there was a significant increase in microcirculation after plasma exposure. There was also an increase in hemoglobin as well as tissue oxygen saturation after the exposure (Borchardt *et al.* 2017). Other optical methods were also used to monitor other factors such as carotenoid and hydration profile after the treatment of CAP. Application of CAP decreased beta-carotene and increased skin surface temperature. This study also proved that CAP influenced skin physiology parameters without damaging the skin or its functions (Fluhr *et al.* 2012). CAP-induced NO was found to enhance the dermal microcirculation in human skin *in vivo*, which was also found to be dependent on the depth in treated skin areas (Heuer *et al.* 2015). When CAP was applied for a duration of 90 s, tissue oxygen saturation and cutaneous blood flow were increased (Kisch *et al.* 2016*a*). The repetitive application of CAP was found to enhance microcirculation for a prolonged period of time. This enhancement was dependent on the number of exposures of CAP (Kisch *et al.* 2016*b*).

CAP is portrayed as an inexpensive but a promising and safe treatment strategy. Previous studies have

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shown that CAP can be safely used both in humans and other animal models. A toxicity study using CAP has shown that treating zebra fish embryos did not induce any morphological defects at shorter duration of plasma exposure (Gandhirajan *et al.* 2021). An *in vitro* study assessed the genotoxic potential of CAP using micronucleus (MN) assay, which is used to test the genotoxicity of different chemical and physical factors. This study showed that there were no newly formed MN post plasma exposure validating the safety of CAP (Bekeschus *et al.* 2018).

Evidences supporting cold physical plasma in wound healing

Laboratory studies

Laboratory investigations have been conducted to yield conclusive evidence that substantiates the notion of the implementation of CAP in the process of wound healing. Table 1 summarizes the different animal models used for study of wound healing. The research conducted by Abbasi *et al.* demonstrated the impact of CAP on burn wounds that were infected with strains of *Pseudomonas aeruginosa*. In this experiment, a circular wound with a diameter of 2 cm was created on BALB/c mice, which was then inoculated with a bacterial suspension of 0.5 McFarland. The mice were subjected to helium plasma treatment for a period of 90 s for 5 consecutive days. Following the 5-day duration of CAP therapy, the burn wound had been fully restored, as the infection had been completely eliminated (Abbasi *et al.* 2021). Another *in vivo* study has been conducted to examine the mechanisms that account for the hastened rate of wound healing through the use of CAP. The results of this study indicate that the use of helium as a feed gas for CAP stimulated the production of endothelial nitric oxide synthase, which is an enzyme involved in the synthesis of nitric oxide in endothelial cells. Furthermore, the study demonstrated that the use of CAP led to the expression of pro-angiogenic factors such as PDGFRβ and CD31 markers and activated VEGFA/VEGFR2 signaling, which resulted in enhanced neovascularization and accelerated burn wound healing in BALB/c mice (Duchesne *et al.* 2019). An additional investigation, which involved 129 Sv/Ev mice, produced similar results, with an increased rate of wound healing observed when the mice were exposed to the CAP device MicroPlaster ß® (argon gas) for a duration of 2 min for 10 days. Following the isolation of the dermal tissues, RT-PCR was performed, and the results demonstrated an increase in mRNA expression of CD31 and FGF-2 markers, providing evidence of accelerated wound healing at the molecular level (Arndt *et al.* 2018). A study showed that female SKH1-hr (hairless, 8–12 week) immunocompetent mice models were treated with CAP, kINPen 11 using argon as the feed gas on the dermal wounds created on both the ears. Repeated CAP treatment for a span of 15 days showed accelerated wound healing independent of the antiseptic effect of the plasma in the mice model (Schmidt *et al.* 2017).

CAP was used as an effective therapeutic strategy for the treatment of diabetic wounds. He *et al.* conducted a study wherein db/db mice and C57BL mice were treated with CAP (helium gas) for varying durations over a period of 2 weeks. The results of this study revealed a noteworthy increase in the rate of wound healing in the treated mice, as compared to the control group, which was found to be independent of the duration of exposure (He *et al.* 2020). Another study investigated the effect of CAP on atopic dermatitis using a mouse model. NC/Nga mice were used for this study and were induced with atopic dermatitis through the use of *Dermatophagoides farinae* extracts. CAP with argon as feed gas was administered to the mice for a duration of 3 min for a span of 17 days. Subsequently, there was a significant improvement in the severity of atopic dermatitis post CAP treatment. Furthermore, it was observed that serum IgE levels decreased in the atopic dermatitis mouse model upon CAP treatment (Moon *et al.* 2021).

Rats and rabbits have been utilized as efficacious models for wound healing due to their robust skin, which closely resembles the wound healing process in humans as compared to mouse models. Additionally, these animals possess a significant surface area on their bodies, making it feasible to generate larger wounds. *In vivo* models, the study conducted by Salehi *et al.* examined the efficacy of CAP as a treatment for chemically induced wounds. The CAP, which was supplied with argon feed gas, was administered to male Wistar rats. The skin samples obtained were scrutinized for markers of oxidative stress, such as catalase, superoxide dismutase, and glutathione reductase. The results indicated that CAP, when directly applied to the skin, had an accumulative effect of oxidative stress in the tissue. The increased level of oxidative stress led to the stimulation of wound reepithelialization (Salehi *et al.* 2015). The kINPen Med system was utilized to evaluate the wound healing rate in Sprague–Dawley rats. The wound site was subjected to the argon CAP for a duration of 14 days, during which the researchers noted a significant increase in the rate of reepithelialization. Additionally, diminished fibrosis and acute inflammation were observed at the site (Breathnach *et al.* 2018). A further examination utilizing Sprague–Dawley rats has demonstrated the effectiveness of argon-based CAP in the context of wound healing. To induce Type I diabetes, streptozotocin was employed, while type II diabetes was induced by means of a high fat diet. The aforementioned rats were subjected to wound creation, followed by exposure to CAP for a period of 120 s over the course of 14 days. A more rapid closure of wounds was observed in rats afflicted with both type I and type II diabetes, and an increased rate of reepithelialization was noted. Subsequent to plasma treatment, levels of antioxidants were measured, revealing an elevation in catalase, glutathione peroxidase (GPx), and superoxide dismutase (SOD) in the treated tissues (Cheng *et al.* 2018).

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Table 1 List of animal models used for studying the effect of CAP on wound healing.

Animal model	Wound type	Plasma discharge type	Results	References
Mice				
BALB/c	Burn wounds	Helium plasma jet	5-day treatment with CAP reduced the bacterial load and the burn wound was completely healed.	Abbasi *et al.* (2021)
BALB/c	Burn wounds	Helium plasma jet	CAP stimulated endothelial nitric oxide synthase as well as activated the expression of pro-angiogenic factors that increased the rate of wound closure.	Duchesne *et al.* (2019)
129Sv/Ev	Dermal biopsy punch wounds	MicroPlaster ß® (argon gas)	CAP improved the rate of angiogenesis and accelerated the process of wound healing.	Arndt *et al.* (2018)
SKH-1	Dermal ear wounds	kINPen Med (argon)	Accelerated wound reepithelialization.	Schmidt *et al.* (2017)
Db/db and C57BL	Diabetic wounds	Helium plasma jet	Increased rate of wound healing independent of the duration of CAP exposure.	He *et al.*, (2020)
NC/Nga	Atopic dermatitis	Argon plasma jet	Significant improvement in atopic dermatitis post CAP exposure along with reduced IgE levels.	Moon *et al.* (2021)
Rats				
Wistar	Chemically induced wounds	Argon plasma jet	CAP was found to induce oxidative stress in the tissues. The increased oxidative stress led to improved wound reepithelialization.	Salehi *et al.* (2015)
Sprague–Dawley	Dermal biopsy punch wounds	kINPen Med (argon)	Increased rate of reepithelialization, along with diminished fibrosis and acute inflammation, was observed post CAP treatment.	Breathnach *et al.* (2018)
Sprague–Dawley	Diabetic wounds	Argon plasma jet	Rapid closure of wounds and increased reepithelialization in rats with type I and type II diabetes.	Cheng *et al.* (2018)
Rabbits				
New Zealand white	Diabetic wounds	Plasma jet	Modifications in the structures of tendons in the joints and reduced count of inflammatory cells were observed in the tendons treated with CAP at 10 kV.	Amini *et al.* (2021)
Larger animals				
Yorkshire pigs	Burn wounds	FE-DBD cold plasma	Safe threshold for plasma exposure at low and high power as well as varying exposure times did not harm tissues, indicating CAP's safety for use.	Wu *et al.* (2013)

New Zealand White rabbits are frequently utilized as a rabbit model for studies involving CAP. Amini *et al.* conducted a study on the efficacy of irradiation with CAP on skin for the purpose of healing injured tissues, such as tendons, which are situated within the body. The induction of diabetes in these rabbits was accomplished by injecting them with aloxan. The wounds were then treated with a CAP device at voltages of 5 kV and 10 kV. The results of this study indicate that CAP effectively modifies the structures of tendons in the joints. Furthermore, it was observed that the count of inflammatory cells was significantly lower in the tendons treated with cold plasma at 10 kV, compared to the control groups and groups treated with plasma at a voltage of 5 kV (Amini *et al.* 2021).

Larger models are utilized to study CAP's impact on wound healing. Bigger models provide more surface area for treatment and shorter exposure times. A study was conducted on Yorkshire pigs to assess the effectiveness of FE-DBD cold plasma. This study found a safe threshold for plasma exposure at low and high power. Moderate energy deposition and varying exposure times did not harm tissues, indicating CAP's safety for use (Wu *et al.* 2013). Sheep has also been used as an important model to study the efficiency of wound healing using CAP. A study conducted on three female Bergamasca sheep by Melotti *et al.* showed that applying helium plasma torch for 2 min followed by injecting peripheral blood mesenchymal stem cells (PB-MSCs) in the wound margins enhanced the rate of wound skin regeneration.

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Macroscopic observations indicated improved wound healing and increased reepithelialization between 28 and 42 days. Combining CAP with PB-MSCs did not cause significant inflammation. The study concluded that this combination could be a promising treatment strategy for wound healing (Melotti *et al.* 2021).

Clinical studies

Various clinical trials and observations have found that CAP treatment exhibits not only antimicrobial activity but also promotes healing in both acute and chronic wounds (Bernhardt *et al.* 2019). Table 2 summarizes the application of CAP in wound healing in patients with different types of wounds. A previous study conducted randomized pilot trials using CAP to reduce microbial load in chronic ulcers. They found that a 2-mi CAP treatment efficiently reduced microbial load and increased the rate of ulcer healing (Isbary *et al.* 2010, 2012). The clinical trials using CAP found to be successful for the treatment of burn wounds, venous ulcers, and chronic ulceration in nondiabetic patients. The repetitive use of CAP was found to promote cutaneous microcirculation and accelerated wound healing by reducing bacterial load (Brehmer *et al.* 2015, Kisch *et al.* 2016*b*). A pilot study demonstrated that using CAP to treat atopic dermatitis reduced its clinical severity and lowered *Staphylococcus aureus* burden (Kim *et al.* 2021). The pressure ulcer scale for healing (PUSH) scale measures pressure ulcer healing over time, distinguishing between healing and nonhealing ulcers. In a clinical trial, treating pressure ulcers with weekly CAP for 8 weeks resulted in significantly improved PUSH scores and less exudate after 1 week. CAP also decreased bacterial load regardless of the bacterial species (Chuangsuwanich *et al.* 2016). A randomized controlled trial showed that CAP enhanced various the rate of wound healing in patients with therapy-refractory chronic wounds. There was also a reduction in bacterial load after treatment with CAP (Moelleken *et al.* 2020).

Superficial skin erosion is a cutaneous medical condition affecting the integumentary system. It can be caused by drugs, skin erosion secondary to eczema, and improper wound treatment. Gao *et al.* conducted a study in which patients with various superficial skin wounds were treated with CAP until complete healing. CAP successfully healed all types of superficial skin wounds without observed side effects (Gao *et al.* 2019).

A case study was conducted on an elderly patient with a chronic wound on his lower right leg. Despite surgery, there was no improvement in the wound. A wound patch and energy supply unit were used to generate CAP, resulting in significant improvement in wound healing and a decrease in *Staphylococcus aureus* colonies (Landscheidt *et al.* 2022).

A 20-year-old female with psoriasis plaques on her left hand was treated using plasma coagulation controller (PCC), which creates plasma using high voltage pulses. PCC was applied for 30 s using the standard operating conditions. The patient did not use any medications for 4 weeks prior to the PCC treatment. Progress of the treatment was checked on day 3, 7, and 14 and significant healing progress was observed. There was a gradual redness, dimensions and scale reduction of the plaque which was observed till the complete disappearance on the 14th day (Gareri *et al.* 2020).

The impact of CAP on various kinds of acute wounds has been extensively scrutinized. A randomized-placebo controlled pilot study was performed wherein argon CAP was utilized on the skin graft donor sites. The subject sites were exposed to CAP and placebo (argon gas) for a period of 2 min. The outcome demonstrated an enhanced rate of wound healing among patients who underwent argon CAP treatment in comparison with those who received placebo (argon gas) (Heinlin *et al.* 2013).

A study was conducted to examine the impact of CAP on a cohort of five individuals who underwent ablative laser lesioning with CO_2 laser. The subjects were uniformly afflicted with four wound areas of comparable dimensions on their left lower arms. The application of plasma treatment utilizing the kINPen MED plasma device was executed on these sites. CAP treatment was administered in 30-s intervals over 3 consecutive days. This regimen was discovered to be adequate in enhancing the pace of wound healing (Metelmann *et al.* 2013).

Another study was conducted on acute surface injuries caused by fractional CO_2 laser exposure. The participants who took part in this investigation were separated into four categories. Two of the groups were subjected to the standard clinical treatments, which included topical ointments and fibroblast growth factor sprays. The other two groups consisted of individuals who were treated with CAP and a control group. The plasma was exposed for a duration of 60 s and placed at a distance of 10 mm from the skin. The study did not find any significant differences between the groups with regard to wound healing. Nevertheless, the treatment with CAP showed a considerable reduction in redness and roughness of the skin when compared to other treatment methods (Nishijima *et al.* 2019).

Burns occur when exposed to heat, chemicals, lasers, radiation, or electricity. This damage affects both the exposed site and the body as a whole. Burn injuries cause changes in capillary permeability (Tiwari 2012). Burn wound healing is dependent on the depth of the burn. Burn wounds are classified based on the depth of the tissues affected by the burn. There are three major types of burn wounds: first-degree burns, second-degree burns, and third-degree burns. The use of CAP has been effective in treating burn injuries (Deodhar & Rana 1997, Tiwari 2012).

Betancourt-Angeles *et al.* conducted a case study that detailed the application of CAP on a 59-year-old patient who had sustained second-degree burns from boiling

Table 2 List of CAP applications for healing of different types of wounds.

Study type	Wound type	CAP device	CAP parameters					Treatment time	Outcome	References
			Frequency	Voltage	Power	FR, slm	Distance[d]			
CT	Chronic ulcers	MicroPlaSter alpha and beta (Argon)	2.6 GHz	50–100 V	86 W	2.2		2-min exposure/day.	Reduced microbial load and increased healing rate of chronic ulcer.	Isbary *et al.* (2012), (2010)
CT	Venous ulcers	PlasmaDerm® VU-2010	50 Hz	230 VAC	8 VA			Average time of 11 min (45 s/cm^2 wound area) per exposure. 3 times a week and for 8 weeks.	Reduced microbial activity and accelerated healing of venous ulcers.	Brehmer *et al.* (2015)
Pilot Study	Atopic dermatitis	MediPL Derm	2.45 GHz ±50 MHz		1.5 W	0.6	5 mm	Plasma exposed for 3 times/day and treated at 0, 1, 2 weeks.	Improved mild and moderate atopic dermatitis and reduced proportion of *Staphylococcus aureus.*	Kim *et al.* (2021)
RCT	Pressure ulcers	Argon Plasma Jet	50 Hz		5[a]; 0.682[b] W/cm^2		5 mm	Plasma exposed at 1 min/cm^2 and once a week for 8 weeks.	Improved PUSH score and reduced bacterial load regardless of the bacterial species.	Chuangsuwanich *et al.* (2016)
RCT	Chronic wounds	SteriPlas® (Argon)	2.6 GHz	50–100 V	86 W	2.2		2-min exposure on the wound site once a week or 3 times a week.	Improved wound healing in patients with therapy-refractory chronic wounds.	Moelleken *et al.* (2020)
CCS	Superficial skin wounds	Plasma Jet		10 kV	50 mW; 5 mA[c]		10 mm	The wound was exposed to CAP for 5 min a day and the procedure was done every 2 days.	Complete healing of the superficial skin wounds irrespective of the type of lesions.	Gao *et al.* (2019)
CCS	Chronic wounds	CPTpatch	NA					2-min plasma exposure 3 times a week, for first 4 weeks and in the next 4 weeks, the plasma was exposed twice a week.	Significant improvement in rate of wound healing as well as reduction in the *Staphylococcus aureus* colony.	Landscheidt *et al.* (2022)
CCS	Psoriasis	PCC	5 kHz	7 kV			1 cm	The psoriasis plaques were exposed to plasma for 30 s for 14 days.	Gradual reduction of psoriasis plaque and complete disappearance on 14th day.	Gareri *et al.* (2020)
RCT	Wounds at donor skin graft sites	Argon Plasma Jet	2.45 GHz		86 W	2.2		The wounds were exposed to plasma for 2 min/day for 7 days.	Rapid healing rate of wounds at skin graft sites.	Heinlin *et al.* (2013)

(*Continued*)

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Table 2 (Continued).

Study type	Wound type	CAP device	CAP parameters					Treatment time	Outcome	References
			Frequency	Voltage	Power	FR, slm	Distance[d]			
CCS	Ablative CO_2 laser lesions	kINPen®MED plasma jet (Argon)	1 MHz	2–3 kV		4–6		CAP was applied 30 s intervals for 3 days.	Improved rate of wound healing and scar recovery.	Metelmann *et al.* (2013)
RCT	CO_2 laser skin wounds	kINPen®MED plasma jet (Argon)	1 MHz	2–3 kV		4–6		The wounds were exposed for a duration of 60 s to the plasma.	Improved wound healing rate with reduction in redness and roughness of the skin.	Nishijima *et al.* (2019)
CCS	2nd-degree burns	Helium Plasma Jet	13.56 MHz		10 W		5 mm	The site of the burn injury was treated using plasma for 180 s. Two treatments were performed in a single day.	Increased rate of reepithelialization with decontamination of bacteria on the wound sites without any inflammatory effects.	Betancourt-Angeles *et al.* (2017)

[a]Power intensity level; [b]Output power measurement; [c]Discharge current; [d]Distance between lesion and device.
CCS, clinical case study; CT, clinical trial; FR, flow rate; NA, not available; PCC, plasma coagulation controller; RCT, randomized controlled trial; slm, standard liter per minute.

oil on their thigh and shin. The treatment employed helium as the feed gas and lasted for a duration of 180 s, with a distance of 5 mm between the target site and reactor. Two treatments were administered on the same day, with a 4-h interval between each treatment. Following the initial treatment, wound epithelialization was observed. Sixteen hours after the second plasma treatment, both wound sites exhibited an increased rate of reepithelialization, and the bacteria in the region of the wound were completely decontaminated. The wound sites experienced no inflammatory effects during this process (Betancourt-Angeles *et al.* 2017).

Limitations of CAP

In the previous sections, we highlighted numerous advantages of CAP and its effectiveness in wound healing. However, CAP also presents with a few limitations as well. The plasma medicine being a newly recognized field, its biological response of CAP is not very well understood because of the complexity and its diverse effects even in genetic, cellular, and tissue levels (Friedman 2020). There are many facets that needs to be brought into consideration for application of CAP for wound healing, especially since there is a need for a constant and set of uniform parameters to regulate the CAP generation as this will be considered as a 'dose' similar to radiation and phototherapies (von Woedtke *et al.* 2020). Another limitation that needs to be considered is the use of different noble gases for the treatment. Several studies have shown the application of using either argon or helium for the treatment of similar types of wounds. The effectiveness of the different gases needs to be compared such that either one of the noble gas or the mixture of gases could be used to produce CAP, such that it can be used as a standard treatment regimen for comprehensive wound care. Currently there are two major types of CAP technologies used. One is the jet-type CAP device and another is the DBD CAP device. The availability of these different plasma devices also holds a major uncertainty based on how effective are these devices in wound healing as each technology uses different parameters to produce plasma. It is necessary to develop the technology that has uniform parameters (gold standard) that will be suitable for conducting uniform clinical trials in plasma medicine. Achieving this will overcome a major hurdle of translating this technology effectively to the clinical environment. Plasma medicine is an evolving field and investigators from physics, biology, and medicine will help address these limitations to develop and improve CAP as a reliable therapy for wound healing.

Conclusion and future prospects

A 'double-edged sword' classically refers to a situation that has both positive and negative consequences or effects. RONS are example of double-edged sword as low levels

initiate physiological signaling mechanism, whereas higher amount leads to oxidative stress and cytotoxicity. Hence, harnessing RONS for medical applications is a challenge due to lack of understanding of the interplay between diverse species of RONS and antioxidants in biological systems. Maintaining a balanced redox state is necessary for energy production, metabolism, and defense against oxidative stress in prokaryotic as well as eukaryotic cells. However, the prokaryotes need lesser exogeneous RONS to disturb their redox homeostasis in comparison with eukaryotic cells. CAP deposits a cocktail of RONS on chronic wounds that are toxic to prokaryotic cells (bacteria) and at the same doses activates eukaryotic cells (fibroblasts, endothelial and immune cells), leading to rapid wound closure. Thus, harnessing RONS for a constructive wound healing application would serve as a double-edged sword.

Several studies have validated the role of CAP and has shown safety and success in the treatment of wide varieties of wounds ranging from acute wounds to injuries caused by burns. However, more studies have to be conducted to further evaluate the effect of CAP treatment in antibiotic synergy, acquired resistance and its potential use in treating other dermal lesions. Even though there are few limitations, addressing these will make CAP therapy more reliable to use. Sustained efforts are needed in the plasma medicine community in this direction to elevate this technology further into the clinic.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the study reported.

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Author contribution statement

Conceptualization, MS and RKG; writing – original draft preparation, RM, RE, JPS, and PS; writing – review and editing, RKG, MS, and SS. All authors read and agreed to the final version of the manuscript.

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