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Review Article

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Overview of Cold Atmospheric Plasma in Wounds Treatment

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Abstract

Cold atmospheric plasma (CAP), a room temperate ionised gas, known as the fourth state of matter is an ionised gas and can be produced from argon, helium, nitrogen, oxygen or air at atmospheric pressure and low temperatures. CAP has become a new promising way for many biomedical applications, such as disinfection, cancer treatment, root canal treatment, wound healing, and other medical applications. Among these applications, investigations of plasma for skin wound healing have gained huge success both in vitro and in vivo experiments without any known significant negative effects on healthy tissues. The development of CAP devices has led to novel therapeutic strategies in wound healing, tissue regeneration and skin infection management. CAP consists of a mixture of multitude of active components such as charged particles, electric field, UV radiation, and reactive gas species which can act synergistically. CAP has lately been recognized as an alternative approach in medicine for sterilization of wounds by its antiseptic effects and promotion of wound healing by stimulation of cell proliferation and migration of wound related skin cells. With respect to CAP applications in medicine, this review focuses particularly on the potential of CAP and the known molecular basis for this action. We summarize the available literature on the plasma devices developed for wound healing, the current in vivo and in vitro use of CAP, and the mechanism behind it as well as the biosafety issues.

Keywords: Cold Atmospheric Plasma, Wound Healing, Plasma Mode of Action, Disinfection, Reactive Species, Chronic Wounds, Acute Wounds

Introduction

Physical plasma can be generated by adding energy (heat or electromagnetic fields) to a neutral gas until it becomes an ionized gas, called "plasma". It reflects the fourth essential state of matter, when enough energy is added to the gas. Plasma composed of reactive chemical species as well as charged species, such as electrons, ions, neutral molecules, and atoms. In addition, radiation emission occurs during plasma generation in the ultraviolet (UV) as well as visible and near-infrared regions (Figure 1) [1].

Figure1: Components of Cold Plasma

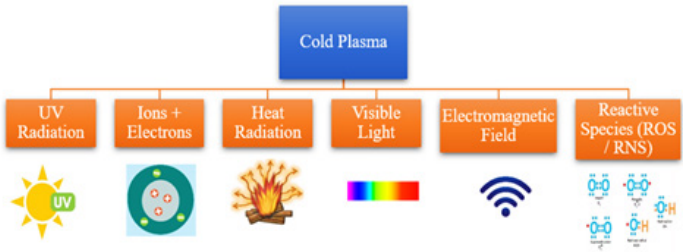

Figure1: Components of cold plasma

Plasma can be classified according to temperature into thermal (hot), and non-thermal (cold). Hot plasma is fully ionized, whereas cold plasma is partially ionized, therefore, besides electrons and ions, there are neutral atoms too. All particles (electrons (light particles),) and atoms and ions (heavy particles)) have the same temperature in thermal plasma, and they are therefore in thermal equilibrium due to thermal motion. However, non-thermal plasma has particles that are not in thermal equilibrium and are generated with a high frequency alternating field in order to obtain "cold plasma" [2,3].

Cold Plasma can be created in the laboratory setting by applying an external source of energy, such us an electromagnetic field, to a gas. This can be achieved at low and atmospheric pressure by using different gases, such as air, helium or argon. These cold atmospheric plasma (CAP) sources can be well controlled and open to air, allowing for the maintenance and application of CAPs with temperatures below 40 oC. Thus, its temperature remains low and is suitable for biological purposes.

Cold Atmospheric Plasma

The development of various devices for medical application of CAP, suggests early adoption of cold plasma as a new tool in the biomedical field. These devices are appropriate in this field because only plasma at a temperature slightly higher than the body temperature is used hence it is adequately safe for human treatment. These plasma devices are often powered by carrier gas argon or helium and are referred to as "cold atmospheric plasma". The interaction of particles at the argon/helium plasma interface with the atmosphere is a known feature of these plasmas. In this situation, CAP application generate a surge of highly reactive species including oxygen species (ROS) such as singlet oxygen (1O2), ozone (O3), superoxide (O2 −), and hydroxyl radicals (OH−), as well as reactive nitrogen species (RNS) such as nitric oxide (NO), peroxynitrite (ONOO−), and nitrogen dioxide (NO2) [4]. These species contribute considerably to the biological effects encountered in cold plasma application and are the focus of comprehensive studies in plasma research.

Recently, new CAP devices with well controlled temperatures below 40°C have been developed for a variety of medical applications. They differ in the technique of plasma production, geometry of the source, working gases, which all lead to the production of plasma with different characteristics and applications. The most utilized technologies used for medical purposes are the plasma jets (Figure 2A) and the dielectric barrier discharges plasma (DBD) (Figure 2B) [5]

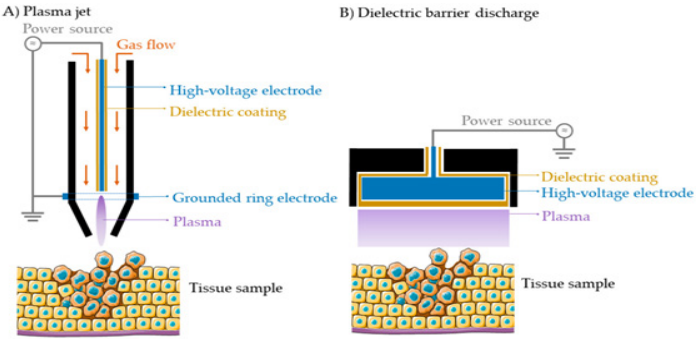

Figure 2: Schematic representation of the two most common plasma devices for medical application. (A) Plasma jet, (B) Dielectric Barrier Discharge Plasma (DBD) [6]

DBD is composed of power supply, two electrodes with a layer of dielectric between them (Figure 1 B). Applying high voltage between the electrodes causes the plasma to appear on the surface of the dielectric material. The exposed tissue in this case serves as an active electrode. In this setup, no voltage is applied directly to the body and most of the energy is deposited in the discharge itself, leaving the exposed tissue unharmed. However, plasma jets generated in open air makes it easier to expose the target at any appropriate distance from the plasma generator and does not require any special contact. As the plasma stream is delivered to the target in a non-contact manner, the risk of tissue adhering to the plasma surgical equipment is eliminated [5].

The development of these devices permit safe plasma application on animal and human tissues which has encouraged various therapeutic application of CAPs and the emergence of plasma medicine technology. This field of medicine aims to exploit the effects of CAP by utilizing the distinct interaction of plasma components with living cells [1]. These interactions may lead to either stimulation or inhibition of cellular function; thus with special tuning and modifications, this technique could be employed for various therapeutic purposes [7]. Cold atmospheric plasma (CAP) devices are currently being developed for a variety of medical applications in oncology, surgery, otolaryngology, gastroenterology, and odontology [8, 9]. However, most clinical studies that have been conducted so far are concentrated in the area of wound healing which has demonstrated high efficacy of CAP in the treatment of chronic and acute wounds. CAP accelerates wound healing in patients by activation of wound healing relevant growth factors and cytokines and by killing bacteria [10, 11]. The objective of this review is to explore the latest evidence of the biological effects, mechanisms of action, and clinical benefits of CAP applications in the wound healing process.

Skin Damage and Phases of Normal Wound Healing

When injured, the skin displays a remarkable ability to repair and regenerate itself through a highly regulated biochemical mechanism. Several cell types play a major role in normal wound healing processes including platelets, neutrophils, macrophages, keratinocytes endothelial cells, and fibroblasts. The regeneration process can be categorized into four coordinated and overlapping phases: haemostasis, inflammation, proliferation, and remodeling.

1. Following injury, platelets adhere to damaged blood vessels and initiate a hemostatic reaction, giving rise to the coagulation cascade that prevents excessive bleeding leading to thrombus formation. Platelets initiate the coagulation cascade by releasing several factors including platelet-derived growth factor (PDGF), transforming growth factors (TGFs), fibroblast growth factors (FGFs) and vascular endothelial growth factor (VEGF) which attract inflammatory cells such as leukocytes, neutrophils and macrophages [12,13].

2. Inflammation occurs within 24 hours of injury, and lasts for 2 weeks or more. The mediators released by platelets (interleukin-1β (IL-1β), IL-6, IL-8, C-C motif chemokine ligand 2 (CCL2) and tumor necrosis factor-α (TNFα)) attract prominent immune cells

such as neutrophils, macrophages and the T-lymphocytes. Neutrophils are the first cells to respond to the platelet mediators [14-18]. They migrate from the circulation, adhere to vascular endothelial cells and subsequently migrate out to the extravascular space, in response to the chemotactic properties of some of the mediators and with the help of cell adhesion molecules (CAMs) [19]. Neutrophils perform a number of critical functions in normal wound healing including direct phagocytosis of bacteria, generation of antimicrobial proteins and generation of ROS and RNS and secretion of elastase and collagenase. Those two enzymes in particular provoke migration of neutrophils as well as removal of damaged structural proteins. Macrophages release various growth factors (PDGF, TGF-β and β-FGF) and cytokines (TNF-α, IL-1, and IL-6) that control cellular proliferation and angiogenesis. Lymphocytes are the last cells to infiltrate wounds and are particularly important in recruiting fibroblasts through the production of IL-2 [20].

3. The proliferative phase begins with fibroblast migration into the wound, a process initiated primarily by the PDGF that has been released by platelets and macrophages [18]. PDGF stimulates fibroblastic proliferation, chemotaxis, and collagenase production. Fibroblasts accelerate wound healing by depositing structural proteins including collagen, leading to the formation of a rudimentary granulation tissue and the extra-cellular matrix [21]. Fibroblasts also produce the matrix metalloproteinases (MMPs) to facilitate movement within the matrix. In the later stages the fibroblasts decrease their proteolytic activity and switch to depositing structural proteins. This step is regulated by two growth factors; TGF-β secreted by both platelets and macrophages, and connective tissue growth factor (CTGF) secreted by the fibroblasts [22]. Protease enzymes released by fibroblasts later help in the process of extracellular matrix (ECM) remodeling. The fibroblasts also secrete chemo attractants and growth factors, which promote angiogenesis
4. Angiogenesis replaces damaged vasculature with granulation tissue. Epidermal cells, fibroblasts, vascular endothelial cells, and macrophages contribute to angiogenesis by the production of βFGF, TGF-β and VEGF [2]. Epithelialization proceeds with the proliferation and migration of the epithelial cells, and is augmented by EGF, keratinocyte growth factors (KGFs) and TGF-α. The cells and the ECM interact closely and continually in a synergistic manner [23].

5. Remodeling succeeds proliferation phase where wound re-epithelization through keratinocytes and ECM deposition by the fibroblasts and endothelial cells occurs. It may continue for a considerable period of time following injury and is primarily a process of connective tissue remodeling that result in wound contraction and scar formation. During this phase, wound scar matures [24] as while collagen and elastin are deposited and constantly reformed as fibroblasts become transform into myofibroblasts. Myofibroblasts adopt a contractile phenotype, and thus are involved in wound contracture [25, 26]. The transformation from fibroblasts to myofibroblasts controls a delicate balance between contraction and re-epithelialization that in part, determines the pliability of the repaired wound [27]. In addition to fibroblast transformation, apoptosis of keratinocytes and inflammatory cells are key steps in the termination of wound healing and the overall final appearance of the wound [28].

CAP Effects on Wound Healing

Wounds can typically be categorized as acute such as abrasions, scalds, burns, or post-operative incisions, and chronic wounds which include diabetic ulcers, venous ulcers, arterials ulcers, and pressure sores. Acute wounds are those in which healing is anticipated to progress through an orderly physiologic sequence of inflammation, proliferation, angiogenesis and remodeling [29]. However, they can develop into a non-healing state and/or become infected, limiting their capability of successfully going through the phases of healing, and therefore can become chronic in nature. Conversely, chronic wound does not heal in an orderly set of stages and often remain in the inflammatory stage for too long with persistent infections and formation of drug-resistant microbial biofilms. This type of wounds is considered to be associated with high morbidity and is usually characterized with high infection load delaying recovery and impeding antibiotic course of treatment [30, 31].

Wound healing is a complicated process involving skin regeneration, revascularization of blood vessels and deposition of collagen and other skin proteins. Although several strategies have been adopted clinically to improve wound healing, but in thus far they are far from perfect [32]. The current prevention and treatment of these wound infections relies on the use of topical antimicrobials that have significant side effects [33,34] . Alternatively, advanced antimicrobial wound dressings serving as a protective barrier from infection provide autolytic debridement, and manage bacteria, which collectively promote wound healing progress. However, they cost over four times as much as conventional wound dressings and their effects are largely limited due to the formation of drug-resistant microbial biofilms, and are often of variable clinical efficacy. Besides wound healing, the risk of wound contamination and ultimately infection development present as another challenge for why wound healing should be a quick and clean process. Hence, there is a huge demand for new approaches to skin disinfection and improvement of wound healing process [35-37]. During the past decade a variety of different CAP devices have been developed and tested for wound healing potential. Numerous effects of CAP in disinfection (bacteria, fungi, and viruses), tissue regeneration (pH modulation, angiogenesis), and anti-inflammation (anti-itch) have been demonstrated in vivo and in vitro experiments described in different parts of the literature [17, 38-42]. Although these effects are affected by CAP design, including energy input, working gas, and exposure time, biological target size and structure are also important considerations. Mixed responses have been reported when using different cells or under different in vivo and in vivo conditions [43].

CAP Inactivation of Wound Bacteria

Several studies have demonstrated the cytotoxic effect of CAP on both bacteria and fungi in addition to its potential as an effective disinfectant. A brief exposure to CAP was effective against an array of common skin bacterial inhabitants including important wound pathogens such as Escherichia coli, group A Streptococcus, Methicillin-resistant Staphylococcus aureus (MRSA), and Pseudomonas aeruginosa, suggesting active involvement in wound healing and disinfection [44]. Effect of CAP was also evident and readily observed in killing of clinically relevant fungal strains in vitro [45]. Moreover, using nails with onychomycosis (a fungal

infection of the nail) as an experimental model showed a significant reduction in bacterial and fungal strains after CAP treatment [46]. Several investigators advocate that CAP technology is safe, effective, and inexpensive therapy that can be used for fungal nail infection treatment. On a follow up to the in vitro study, an in vivo pilot study to evaluate the efficacy of the CAP treatment on 19 study participants with toenail onychomycosis [47]. No long-term sequelae have been observed after CAP treatment, and overall clinical recovery was observed in 53.8% of participants, whereas mycological recovery was observed in 15.4% of participants. A prospective randomized controlled study including 37 patients with herpes zosters (a painful skin infection caused by the varicella zoster virus) revealed that a weekday five-minute CAP treatment is safe, painless, and effective, improving initial healing of the herpes zoster lesions [48]. Taken together, promising effects of CAP have been shown with regard to disinfection with no evidence for resistance of microorganisms. Moreover, CAP wound healing potential has gained much attention lately because it offers noncontact, painless and cheaper alternative to a wide variety of wounds including ulcerations and burn wounds.

CAP Stimulation of Wound Healing

Scientists endeavor in this area focused on assessing and evaluating the biological factors that play a major role in wound healing process and that are elicited by CAP exposure. In an in vitro experiment using fibroblasts and aiming at identifying the pro-inflammatory factors upon exposure to CAP, IL-6 and IL-8 were readily available in addition to TGFβ1/2 which becomes abundant collectively during the first phase of inflammation [49]. Protease inhibitor, Serpin E1, a tissue remodeling regulator and CD154 that promotes leukocytes recruitment to the lesion were all identified as inflammatory phase factors during wound healing. Presence of the later regulatory factors help to improve immunity required to fight infection at the scene demonstrating a very well-orchestrated sequence of cellular process including build up and defence. In support of these in vitro findings and parallel to the results presented earlier, in vivo studies have shown that macrophages and neutrophils accumulate in the same sequence during the early phase after CAP treatment [50].

Diabetic foot ulcers

Diabetic foot ulcers are common and recurrent complication of diabetes which is slow healing with infections that frequently develop resistance to most common antibiotics used [51-53]. Several studies have been conducted comparing the effect of using CAP to treat diabetic foot ulcers with bacterial burden as oppose to the presently used management schemes of treatment. CAP proved to be effective in different aspects regarding diabetic foot ulcers with shorter wound closure time, improved microcirculation and granulation and decreased bacterial overall load [54]. Wound healing improvement in most cases is independent of bacterial load or infection status emphasizing the divergence of the two processes. Impact on infection status concomitant to CAP treatment is not very noticeable explaining that the two mechanisms are separately controlled.

Pressure ulcers

Also named as bed sore and is readily affecting life style and quality. They are localized injury to the skin as a result of pressure or pressure with shear combination. A mouse model was used to evaluate CAP potential in treating pressure ulcers as oppose to control untreated animals [55]. Comprehensive examination of the morphology changes of the healing wound demonstrated progressive re-epithelization of the affected area with elongated exposure to CAP. In addition, histological studies and follow up showed continuous and enhanced angiogenesis at the site of exposure. CAP has proved effective in normalizing skin texture and elasticity due to more collagen deposition. Comparing the results of the exposed to the unexposed animals, wound healing was faster and wound re-opening was very much less likely to happen which with special attenuation presents a potential therapeutic application.

Burns wounds

Growing research on mouse models with second and third degree burns have demonstrated that CAP accelerated wound healing and reduced pain and itching at the site. On further investigation, CAP healing effect was mediated through promoting angiogenesis via the recruitment of VEGF and other growth factors. Nitric oxide (NO) formation during the process of healing accelerated the recruitment of several pro-angiogenic factors including TGFβ1/2, platelet-derived growth factor receptor beta (PDGFRβ) [56,57]. In a follow up of earlier experiments it was demonstrated that CAP treatment accelerate chemical burns healing by a similar mechanism that is based on emitting a surge of free radicals that augmented the re-epithelization process and prevented wound infection [58].

Mode of Action of CAP

In vitro [59] and initial clinical studies of chronic wounds in animals [60,61] and humans [62-65], have shown that physical components such as UV radiation or electrical field as well as chemical components such as ROS and RNS could act independently or in synergy in CAP treatments [66-69]. Despite it is hard to determine which CAP component has the most significant impact [70, 71], ROS and RNS seem to play a key role in the interaction between CAP and cells (prokaryotes and eukaryotes). Previous clinical experiments show efficacy for CAP treatment of chronic and acute wounds in patients without any significant side effects on normal tissue. These wound healing effects are a result of two processes; antiseptics effects in combination with promoting wound healing [42, 43].

Mechanism of Plasma-Induced Antiseptics

CAP was found to inactivate effectively broad-spectrum of infectious microorganisms within minutes of exposure without causing allergic skin reactions or counteractions of the plasma applications [72]. However, the exact biophysical mechanisms of cold plasma-induced bacteria death are still not fully explored. Generally, as CAP sources are operated at ambient pressure in contact with air, a number of microbicidal plasma-generated agents including ROS, RNS, UV radiation, energetic ions, and charged particles are generated. ROS and RNS species are the main agents involved in the antimicrobial effects [71, 73]. The two other, non-RNOS sources (UV and electric field) generated by CAP don't have direct contribution to the microbicidal effect. Nonetheless, this does not exclude a possible indirect contribution by the formation of ROS in the gas phase that can inactivate microorganisms by attacking different microbial structures in various ways (Figure 3) [74].

1. 1- electroporation- and oxidation-induced cell wall/ membrane damage leading to leakage of cellular components. Reactive oxygen components strike the microbe surface, triggering cell wall tension leading to mechanical rupture and subsequent leakage of cell content [75, 76].

2. 2- Intracellular oxidation caused by induction of intracellular reactive oxygen radicals. Singlet oxygen (1O2), atomic oxygen (O) and even ozone (O3) generated by CAP can diffuse across cell membranes, leading to the formation of other reactive radicals such as hydroxyl (OH) and superoxide (O2•−) free radicals. Free radical species have a strong oxidative effect on intracellular components that can potentially cause damage to cellular macromolecules such as lipids, proteins, polysaccharides, leading to disrupting cellular metabolism [78].

3. 3- Direct DNA damage causing double-strand breaking, affecting cellular replication triggered mainly by reactive species. The reactive species that participate in DNA fragmentation include both free radicals and non-radical species [70, 77].

4. 4- Induction of programmed cell death (PCD) in bacteria, called bacterial apoptosis-like processes, is being discussed recently as a potential mechanism of CAP-induced bacteria inactivation [79]. This process is a genetic program cell death that generates rapid cell death via pathways similar to intrinsic pathway of apoptosis in eukaryotes [80].

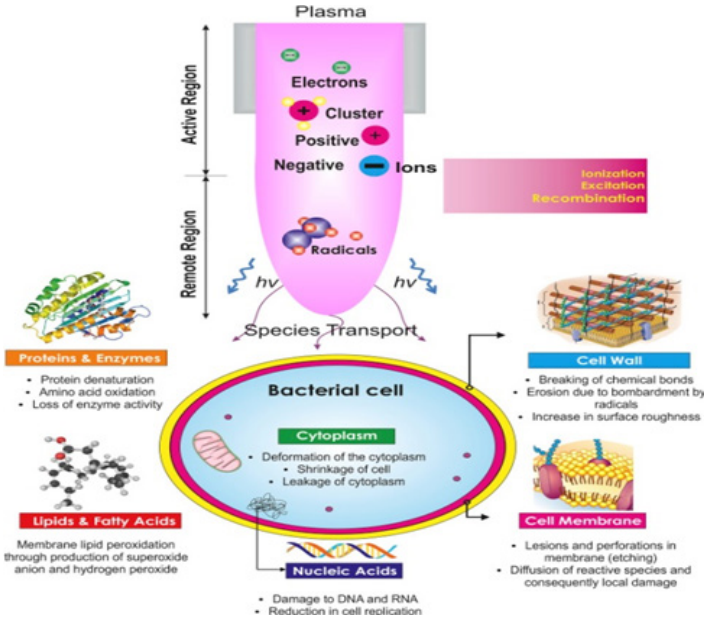

Figure 3: A schematic of the mechanism of cold plasma induced-antiseptics.

In conclusion ROS/RNS generated by CAP have direct interaction with macromolecules in the cell wall and plasma membrane, leading to the observed bactericidal effect and dsDNA fragmentation [81-84]

Mechanism of CAP-stimulated Tissue Regeneration

In contaminated wounds, CAP-induced antiseptics has an important additional effect on cells involved in wound healing due to keratinocyte and fibroblasts proliferation and migration, increased cutaneous microcirculation and monocyte stimulation [43,85]. Conversely to the antimicrobial effects which require both ROS-RNS composition resulting from plasma generation under atmospheric air conditions, the repair mechanisms on mammalian cells is mostly dependent on ROS (Figure 4) [5].

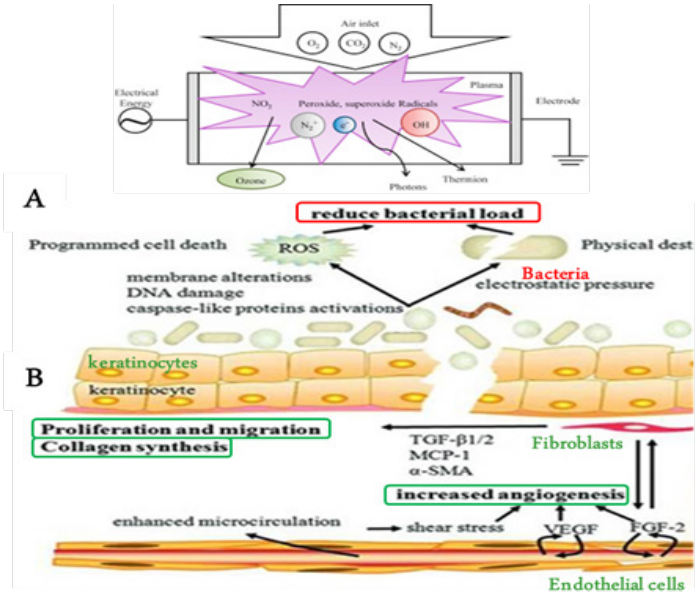

Figure 4: Schematic illustration of a general summary of CAP therapeutic mechanism on wound healing. CAP-derived reactive species inactivate pathogenic bacteria (A) as well as promote fibroblast proliferation and migration, improve angiogenesis, and increase local microcirculation (B).

ROS act as a secondary messenger to many cells involved in the repair process, and appear to be important in coordinating the recruitment of immune cells to the wound site and effective tissue repair. ROS also possess the ability to regulate the angiogenesis at the wound site and increase local microcirculation. Therefore the manipulation of ROS represents a promising avenue for improving wound-healing responses when they are stalled. By admixing molecular gases, the composition of reactive species in the plasma effluent can be modulated. Addition of N2 leads to increased generation of RNS [86], whereas addition of O2 shifts the balance more toward the ROS [87].

The second stage of wound repair is related to cell proliferation and migration as well as angiogenesis. CAP stimulates keratinocyte and fibroblast, resulting in faster cell proliferation and migration which can shorten the wound healing process [88]. This is through activation of cytokines and growth factors synthesis like transforming growth factors (TGF-ß1/2), and alpha-smooth muscle actin (α-SMA) as well as type I collagen [89]. Moreover, monocyte chemoattractant protein 1 (MCP1) is increased early alongside IL-6 while TGFβ1 and 2 are over-expressed in the later phase emphasizing the sequential effect of CAP on the wound healing process. Continuous fibroblasts migration results in accumulation of ECM proteins although less evidence is available to support CAP role in fibroblast proliferation and differentiation [90, 91]. Furthermore, UV radiation and free radicals generated by cold

plasma affect DNA therefore, introducing changes in cell proliferation pathway [77].

CAP and by generation of different ROS and NO species, is capable later to stimulate angiogenesis. Growth factors (VEGF, EGF, FGF), cytokines (e.g. IL-1, 2, 6, 8; TNF, TGF) as well as ROS and NO have important role in the angiogenesis phase of the normal wound healing. Hydroxyl radicals and hydrogen peroxide generated by CAP increased endothelial cell proliferation by release of growth factors like VEGF and IL-6 that are involved in cellular proliferation pathways as well as release of FGF-2, which is a promoter of angiogenesis [90] or by production of Nitric Oxide (NO) [56]. CAP produces copious amounts of NO that are capable of promoting cellular pathways that help to stimulate wound healing and reduce ulcers inflammation. This is through the activation of the MAPK pathway that is involved in the inflammation and proliferation process via TGFβ1 which is essential for wound healing [92]. These cellular processes include angiogenesis, tissue remodeling, matrix deposition and various growth factors recruitment. Besides growth factors, cytokines, ROS and NO angiogenesis are fundamentally influenced by adhesion molecules, especially integrin expression. CAP is known to modify integrin expression on endothelial cells that mediate binding of cells to components of the ECM facilitating cell migration.

CAP works also by influencing microcirculation. Plasma application in vivo led to a fast increase in dermal microcirculation parameters such as capillary venous oxygen saturation which improve vascular shear stress contributing to new angiogenesis. New vascular networks and enhanced capillary blood flow increase local oxygen saturation and nutrient supply, which also contributes in wound healing [93, 94].

In addition, the immune system also plays an essential role in the wound healing process. In vitro studies have indicated that short-term plasma treatment produces considerable stimulating effects on immune cells to proliferate and function actively which is in support of the antimicrobial defense in removing pathogens. CAP has been hypothesized to act through quenching the inflammatory reactions by decreasing inflammatory cytokines secretion and increasing anti-inflammatory mediators such as TGF-β1 and IL10 [95].

Moreover, a study carried out by Anke Schmidt et. Al. [96], to investigate the hypothesis that wound healing is subject to redox control which is provoked by CAP treatment. This study identified a redox regulatory factor Nrf2 that is capable of controlling the redox state of the cell by controlling redox systems like the glutathione peroxidase/ glutathione anti-oxidant pathway. Moreover, the same result has demonstrated the pivotal role P53 displays in controlling angiogenesis and the regulation of nitric oxide synthase (NOS) [97]. P53 activity is especially important during the first stage of wound heling to mediating programmed cell death and promoting cell proliferation during the angiogenesis process [98]. The p53 cascade should be a major hub of cold plasma–cell interactions in keratinocytes. The authors also consider that the upstream serine- (ATM) and serine/threonine-protein kinase (ATR) redox sensors have higher activity, and that MAP kinase signaling should modulate the p53 signals in governing the cellular response towards CAP-derived ROS/RNS (Figure 5) [99].

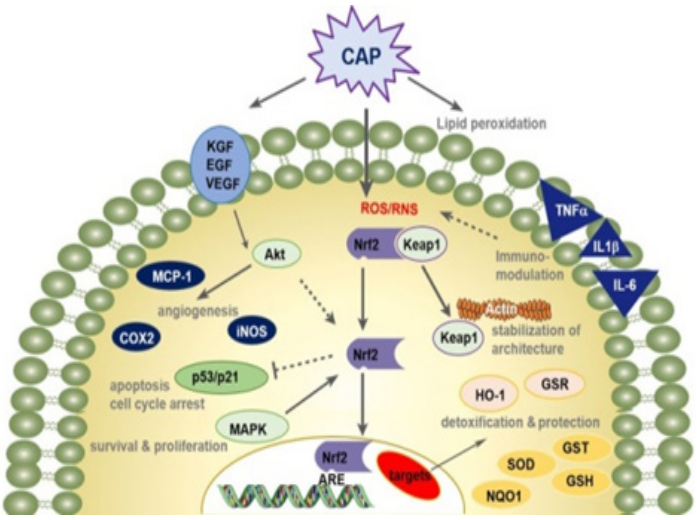

Figure 5: CAP wound healing processes via redox-regulated pathways. CAP Enhance wound healing by involving inflammation, activation of pro-angiogenetic factors, and balance of cellular proliferation and apoptotic events (Von Woedtke, T., 2019) [5].

On the other hand, Biological systems including cells and tissues have established specific mechanisms enabling them to control the amount of reactive species to stabilize a baseline level of reactive oxygen intermediates tolerable and beneficial to the cell [100,101]. Repairing tissues have powerful cytoprotective "resilience" machinery for protection against ROS caused damage [102]. Moreover, recent study shown that ROS play a pivotal role in the orchestration of the normal wound-healing response and possess the ability to regulate the formation of blood vessels (angiogenesis) at the wound site and the optimal perfusion of blood into the wound-healing area [103].

Despite the potential CAP is possessing in wound healing and wounds disinfection, there are still many issues remaining regarding the biochemical and molecular mechanisms by which CAP is eliciting its effect on biological tissues in vivo. Parallel to the studies in vitro, accumulating results on different types of plasma sources and various working parameters and protocols have produced inconsistently variable results accentuating the need for more standardized research with regard to both the technology used and the biological specimens.

Generally, several aspects of CAP may contribute to an improved wound healing including UV radiation and reactive gas species (i.e., ozone) disinfect the wound, generation of nitric oxide (NO) and other or nitrogen species (NOx) stimulates the regeneration of tissue, and electric current stimulates angiogenesis. Furthermore, CAP leads to the decreases of pH of the aqueous medium which may lead to wound acidification, and consequently promotes the healing process [104-107].

Conclusions

In vivo and In vitro studies have demonstrated that CAP affects positively the different stages of wound healing due to the synergistic effect of biologically active plasma components, such as UV radiation and several free radicals species. CAP treatment results in effective inactivation of a broad spectrum of microorganisms

including multidrug-resistant pathogens during the first stage of wound healing and stimulation of skin-related cell proliferation, migration and angiogenesis in the second phase of wound healing. Most importantly, CAP's less intense effects enable its direct application to cells and tissues without any significantly observed side effects making CAP treatment less invasive and stressful for patients. CAP has a bright future due to the possibility of becoming an effective alternative to antibiotics particularly in combating bacterial strains that have developed antibiotic resistance, resulting in eradication of skin and wound pathogens. Another great advantage of CAP-generating devices is their relatively low manufacturing costs, so that accessible, efficient, and cheaper CAP devices can very much likely help to reduce the inflated financial burden imposed on the health budget due to traditional treatments. Overall, this exciting field of plasma medicine is expanding and accumulating evidences mounting supporting the progressive and optimistic use of CAP as a treatment option not only for subcutaneous but also for internal structure. Continuing efforts in this emerging and highly dynamic field of plasma medicine will be necessary to fully understand its mechanisms of action and through standardizing protocols and parameters (temperature of the plasma, duration of exposure, frequency of exposure and distance between the object and the plasma beam) for the safe and effective application of plasma treatment paving the way for a new form of cheap and affordable therapy.

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