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SKIN THERAPY USING COLD ATMOSPHERIC PLASMA

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Abstract: Background: Maintaining skin integrity is essential for protecting against external agents, microorganisms, and dehydration, while also serving social and aesthetic functions. Recent advancements in skin care have introduced innovative technologies, including Cold Atmospheric Plasma (CAP), which shows promising results in dermatology. **Specific Background:** CAP technology, based on physical principles, has emerged as a novel approach in skin treatments, offering potential benefits for aging prevention and skin care. This interdisciplinary field encompasses physics, biology, chemistry, and biochemistry, presenting a complex landscape. **Knowledge Gap:** Despite its potential, there is limited comprehensive analysis of CAP's interactions with skin, its effects, and its practical applications in cosmetic dermatology. **Aims:** This research aims to provide a broad survey of CAP's interactions with skin by discussing the basic structure and functions of the skin, the foundational principles of CAP, and its physical and chemical properties. The study will analyze CAP parameters to highlight known effects and explore potential applications in skin treatment. **Results:** The review reveals that CAP exhibits versatile applications in dermatology, including direct treatment of superficial lesions, indirect treatment via plasma-activated media, and combined use with other therapies. CAP has shown promise in optimizing intact skin, facilitating transdermal drug delivery, and minimizing side effects. However, challenges remain in integrating CAP into routine clinical practice. **Novelty:** This overview synthesizes current knowledge and emerging potential of CAP technology in skin biology, offering a comprehensive evaluation of its effects and applications. **Implications:** The findings underscore CAP's potential to revolutionize skin care and dermatological treatments. Addressing the existing challenges and expanding research could enable CAP to become a valuable tool in both therapeutic and cosmetic dermatology.

Keywords: Skin, CAP, Dermatology, Plasma, Aging.

Introduction

The skin is the largest organ of the human body and ensures several distinct functions because of its particular position, in connection between the outside and the inside of the body. This keratinized tegument envelops the whole body and protects it from environmental aggressions and from massive water loss [1]. Although the physical barrier against environmental and pathogen insults is the main role of this organ, the skin possesses several other functions such as vitamin D productio [2],humidity, temperature and mechanical sensing [[3], [4], [5]], temperature regulation [6], molecule absorption [7], excretion and secretion [[8], [9], [10], [11]] and some

immunological functions [12]. In view of the above, daily cares of this organ are necessary to preserve its integrity and functions. Skin care practices are not only needed for the whole body health but also for social and aesthetic purposes. Cosmetic skin care was practiced from the dawn of time by the ancient civilizations. In the 21st century, with the increase of span life, people from all walks of life ask to live healthy and look younger. Consequently, the global consumer demand of cosmetic products is rapidly expanding today. In 2018, the value of the global cosmetics market was 508 billion U.S. dollars. The market is projected to value at about 758 billion U.S. dollars by 2025 [13]. Modern skin treatment-offer ranges from chemical product application to physical treatments. Among the treatments, creams, sera and oils are commonly used as at-home beauty treatments while skin peeling treatments are often administered by professional beauticians. Physical treatments are also administered in beauty centers although today some devices can be bought for domestic use. Among them, LED lights and lasers are often used for rejuvenation purposes. These light sources stimulate skin renewal by physically removing the external layers of the skin (resurfacing) thus activating skin cell metabolism [14,15]. For a deeper skin rejuvenation, more invasive and expensive techniques such as aesthetic surgery are required. Currently, a physicochemical approach, based on ionized gases, is joining the skin non-surgical treatments. This technology, named Cold Atmospheric Plasma (CAP), was already used in dermatology to promote wound healing. Today, CAP is entering into the cosmetic field, thus providing a new challenge. In reason of their unique ability to generate a complex chemical mix and thanks to their physical properties, CAPs could be a promising alternative in non-invasive treatment of skin. However, the scientific bases of cold plasma effects on skin and the identification of their exact mechanisms of action, both at the cellular and at the molecular levels, are still lacking and they constitute a new active field of investigation. In the present review, based on skin fundamental notions and on chemo-physical properties of plasmas, we described the possible benefic interactions between CAPs and skin and how they could participate in improving skin wellness and regeneration. **The aim of the study:** This research aims to provide a broad survey of the interactions between CAP and skin. , giving some basics about the structure and functions of the skin related to its basic functions, and the main fndouations of cold plasma and its physical and chemical properties.

Methods

1. **Literature Review:** This study conducted a comprehensive review of existing literature on the skin's structure, functions, and the applications of Cold Atmospheric Plasma (CAP) in dermatology. Key sources included scientific journals, clinical studies, and recent reviews on skin physiology, CAP technology, and its impact on skin care and cosmetic treatments.
2. **Analysis of CAP Properties:** The review examined the physicochemical properties of CAP, including its ability to generate reactive species and its effects on skin cells. This involved analyzing studies that describe the interaction of CAP with skin tissues, focusing on its potential to enhance skin rejuvenation and healing through non-invasive means.
3. **Evaluation of Cosmetic Applications:** The research evaluated current and emerging applications of CAP in the cosmetic field, assessing its effectiveness compared to traditional treatments. This included a review of clinical trials and case studies that highlight the benefits and limitations of CAP for skin care, with an emphasis on its potential role in non-surgical skin treatments and aesthetic improvements.

Results and Discussion

Results

2. sagging skin Optimising Intact Skin Using CAP

2.1 sagging skin

if you've spent hours in the gym trying to lose weight, you probably know that saggy skin can be an all-too-common side effect. Saggy skin, on both the face and body, is often associated with the loss of fat. The deterioration or reduction of collagen and elastin in the dermis are another cause of saggy skin. While anyone can get saggy skin, it's more likely to occur in people as they age. People who have lost significant amounts of weight are also more susceptible. Certain medical conditions may also be the cause. Sagging skin can be challenging to treat at home, but there are skin-tightening options that can help, from over-the-counter products to surgical solutions.

What causes saggy skin?

Ehlers-Danlos syndrome is a group of disorders that mostly affect your skin, joints, and blood vessels. People with EDS often have very flexible joints and stretchy skin that bruises easily. Connective tissue is one of your body's basic building blocks. It gives you strength, support, and structure for everything from your skin to your organs. When there's a problem with it, such as with EDS, the effects can be serious. For some, the condition is mild; for others, it's more severe. While there's no cure, usually only one kind -- vascular EDS -- is life-threatening. Firm skin can stretch and snap back into place easily. When skin loses this ability, it starts to sag. Saggy skin can happen almost anywhere on the body. Common areas where you might see saggy skin include:

- eyelids
- jowls
- chin
- throat
- upper arms
- stomach

There are several causes of saggy skin. They include:

Aging:

As skin ages, it loses two important proteins manufactured in the dermis — elastin and collagen. As its name suggests, elastin gives skin elasticity. It provides firm skin with the ability to bounce back when stretched. Collagen is produced by fibroblasts. When skin is taut and firm, it has collagen to thank. Collagen is comprised of tightly-constructed fibers, which help skin maintain its structure and firmness. Both elastin and collagen production decline as people age. These two proteins can also become deteriorated by external factors over time, such as:

- UV exposure
- pollutants in the environment, including cigarette smoke
- certain lifestyle factors, such as poor nutrition and drinking alcohol to excess

Too much sun exposure and not taking care of your skin or health can speed up the process of skin aging. This can make your skin look saggy and wrinkled at a younger age.

Weight loss:

Carrying extra weight for an extended period of time can cause damage to the collagen and elastin fibers in your skin. This makes it harder for skin to snap back when you lose weight. If you lose a significant amount of weight of 100 pounds or more, significant amounts of saggy skin may result. Sagging skin is more likely to occur when weight loss is rapid, such as after bariatric surgery. In some instances, these weight loss procedures may result in large amounts of sagging, drooping skin that hangs on the body. Since younger skin bounces back more readily, your age at the time of weight loss can also play a role in how saggy your skin becomes.

Pregnancy:

Acquiring some degree of saggy, loose skin is common after pregnancy. Women who carry multiples, such as twins or triplets, may see more sagging skin around the abdomen than those who carry one baby. Maternal age may also play a role.

Illness:

There are a few medical conditions that are marked by saggy skin. One of these is a very rare subtype of cutaneous T-cell lymphoma, known as granulomatous slack skin.

People with this condition see a very gradual slackening of skin on the elbows and knees. Saggy skin caused by granulomatous slack skin does not typically respond well to treatment.

Ehlers-Danlos syndrome:

Another condition that causes saggy skin is Ehlers-Danlos syndrome (EDS), a rare, connective tissue disorder that is inherited. People with EDS have a defect in collagen production that results in saggy, doughy skin, often on the face.

Treatment options for saggy skin:

If you are concerned about area of saggy skin, there are things you can do to reduce or eliminate it. Saggy skin amounts can range from slight to significant. When deciding on treatment options, consider these factors:

- the areas of the body where sagging occurs.
- the amount of sagging.
- your feelings about your condition.

Clinical Anatomy of Human Skin:

Human skin is a unique structure, with a surface area of 2 m2 and makes up for up to 20% of the total body weight. Skin is multifunctional as it provides protective barrier, acts as sensory receptor, transports nutrients and metabolites, helps regulate body temperature, and exercises immunological activity. Skin is composed of three layers, from superficial to deep: the epidermis, dermis, and hypodermis. (Figure 2).

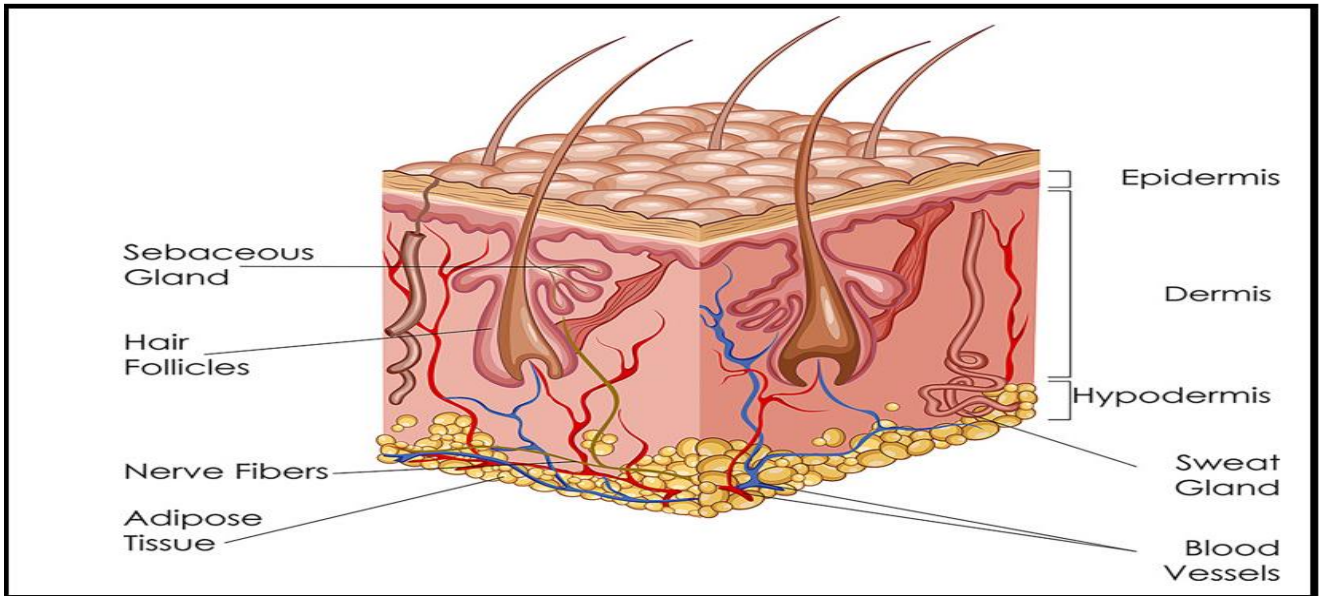

Figure 2: Schematic diagram of the skin: its three layers and accessory structures.

The epidermis is further divided into five sublayers: stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale. The basic cell types of epidermis include keratinocytes, melanocytes, and Langerhans cells. Keratinocytes are of ectodermal origin and have the specialized function of producing keratin. Melanocytes derive from the neural crest and synthesize

melanin. Langerhans cells are dendritic cells and can recognize, process and present antigens to lymphocytes during hypersensitivity reaction.

The dermis is connected to the epidermis through the basement membrane zone. The dermis is divided into two areas: papillary region (superficial area) and reticular region (deep area). It contains adnexal structures, such as sweat glands, hair follicles, sebaceous glands, and nails. The principal component of the dermis is collagen, particularly type I collagen, which is the major stress-resistant substance of the skin.

The hypodermis, or subcutaneous tissue, consisting of loose connective tissue and adipose tissue, attaches skin to the underlying muscles and bones. Here, the main cell types are fibroblasts, macrophages, and lipocytes. The hypodermis also contains larger blood vessels and nerves which supply the skin.

2.2 The Effect of CAP on Skin Cells:

There have been several in vitro studies in the last few years focusing on the effect of CAP exclusively on non-diseased skin cells .These projects mostly used HaCaT human immortalized keratinocytes as the cellular model. Although there were some variations in plasma-induced cellular changes, it was found that the respective change at the cellular level was also reflected correspondingly at the protein level and gene level.

In a relatively early study, Arndt et al. used the MicroPlaSter β plasma torch system on primary human skin keratinocytes (30). Their main novel finding was that antimicrobial peptides of the β-defensin family were upregulated after CAP treatment. Defensins are small cysteine-rich cationic proteins that are active against bacteria, fungi and viruses. They could be found in inflammatory skin conditions such as atopic dermatitis. CAP also induced gene expression of key regulators for inflammation and wound healing, such as interleukin 8 (IL-8) and transforming growth factor beta (TGF-β). However, the proliferation, migration and apoptosis of keratinocytes were not altered by CAP. Subsequent studies using HaCaT cells found different results, as cell proliferation, motility and apoptosis could all be changed using CAP treatment (25–28).

Choi et al. used a low-frequency argon plasma device based on the dielectric barrier discharge (DBD) technology (25). At the cellular level, keratinocyte proliferation was stimulated by CAP, as the transition from G1 cell cycle phase to S and G2 phases was quickened. At the protein level, CAP not only dispersed E-cadherin-mediated cell-to-cell interactions but also translocated β-catenin from the cytosol to the nucleus. At the gene level, CAP treatment increased the expression and transcription of c-MYC and cyclin D1. Therefore, these results of enhanced epidermal cell growth demonstrated that CAP might be used a novel skin regenerating apparatus.

On the contrary, a series of studies conducted by Schmidt et al. using kINPen 09 atmospheric argon plasma jet showed suppressive results instead. Firstly, reduction in cell proliferation, transient increase in cell migration, and secretion of immunomodulatory signal proteins were found in human keratinocytes after direct CAP exposure (26). The above changes were centred around the p53 regulatory axis, with upstream phosphorylation of ATM and ATR and downstream activation of checkpoint kinases Chk and mitogen-activated protein (MAP) kinases. Thus, plasma-induced proapoptotic, proinflammatory, and pro-survival effects were confirmed. Secondly, periodic, long-term, and indirect treatment using plasma-activated medium (PAM) repressed keratinocyte motility and enlarged cell size (27). The authors discovered differential expression of 260 genes involved in inflammation and redox homeostasis using transcriptomic microarray. The protein products of these deregulated genes include various cytokines, growth factors, antioxidant enzymes, and apoptotic signalling targets. Lastly, the same research group changed reactive species generation of the plasma

effluent by modulating the ambient gas composition from pure nitrogen to pure oxygen (28). The oxygen-shielding plasma provided stronger apoptotic effect than the nitrogen counterpart and induced keratinocyte response more efficiently. Gene expression analysis revealed induction of signalling and communication proteins such as immunomodulatory interleukin 6 (IL-6) and several antioxidative molecules.

In summary, the inconsistency in plasma-induced cellular change in healthy skin cells, such as cell proliferation, cell migration, and cell apoptosis are multifactorial. CAP-generating technology (e.g., DBD or others), treatment modality (e.g., direct jet or indirect PAM), plasma parameters (e.g., duration and gas composition), and target skin cell type (e.g., keratinocytes vs. fibroblasts, human vs. murine) all could be contributing factors. More dedicated studies are required to expand our understanding on the effect of CAP on healthy skin cells.

2.3 The Effect of CAP on Skin Histology:

The above CAP-induced cellular effects have been further examined at the histological level. Additional architectural information was obtained using various histological staining methods including haematoxylin and eosin (H&E) staining, Masson's trichrome staining, and immunohistochemical (IHC) analysis. The two consecutive studies published by Choi et al. revealed that short period of CAP treatment on HRM2 mice caused significant increases in epidermal thickness and dermal collagen density (25, 31). Repeated CAP treatment not only caused epidermal expansion by activating β-catenin in the epidermal cells, but also accelerated dermal remodelling. On the other hand, plasma increased the skin tissue expression of various growth factors, such as TGF, vascular endothelial growth factor (VEGF), granulocyte macrophage-colony stimulating factor (GM-CSF), and epidermal growth factor (EGF). Interestingly, Hasse et al. brought more clinical value as they used human skin biopsies from nine patients for plasma treatment (32). Unlike what was shown previously where plasma-generated species only penetrated the first few layers of the epidermis (33), the pro-proliferative effect of CAP in the current work occurred as deep as the stratum basale, while the epidermal integrity and keratin expression remained unchanged. Collectively, these in vivo studies support CAP as an innovative tool with anti-aging potential for the skin.

2.4 The Effect of CAP on Cutaneous Blood Vessels:

in general, CAP can improve cutaneous microcirculation of intact skin. In 2016, Kisch et al. organized a controlled, prospective cohort study including 20 healthy patients using skin at the radial forearm (34). Their DBD CAP device increased cutaneous tissue oxygen saturation by 24% and maintained effective for at least 8 min. In addition, the cutaneous capillary blood flow increased by 73% and remained fast for 11 min. Furthermore, even more prominent elevation of these parameters was found in patients with lower body mass index (BMI).

In comparison, Borchardt et al. conducted a similar study with a smaller number of patients, but assessing lot more circulation parameters for a longer monitoring period (35). Firstly, tissue oxygen saturation and blood flow both improved in a plasma duration-dependent manner. Secondly, skin pH decreased by 0.3 after CAP treatment, whereas skin temperature and moisture were not altered. Most importantly, CAP-related enhancement of skin microcirculation was found to be specific to the plasma treatment and not a result of the applied mechanical pressure.

In order to study the influence of CAP on vascularization-involved molecules in skin-related cells, Arndt et al. performed in vitro and in vivo experiments on a microscopic scale using the MicroPlaSter β plasma torch system (36). CAP significantly activated the expression of artemin, EGF, endocrine-derived VEGF (EG-VEGF), endothelin-1, basic fibroblast growth factor (bFGF), IL-8 in human epidermal keratinocytes, angiogenin, endostatin, monocyte chemoattractant protein (MCP)-1,

matrix metalloproteinase (MMP)-9, tissue inhibitor matrix metalloproteinase (TIMP)-1, and VEGF in human dermal fibroblasts, as well as angiopoietin (Ang)-2, angiostatin, amphiregulin, endostatin, bFGF and angiogenic receptors in human umbilical vein endothelial cells (HUVECs). It was concluded that CAP modulates angiogenesis-related factors in an autocrine and paracrine mode.

2.5 The Effect of CAP on Skin Chemical Components:

The lipids of the stratum corneum is one of the most important components of the skin barrier. Marschewski's group was one of the first to use X-ray photoelectron spectroscopy (XPS) to analyse CAP-induced changes in the physiological skin lipid composition (37). Using stripped off skin lipids from human forearms, the authors revealed that the total carbon amount reduced from 84.4% to 76.7, oxygen increased from 10.8% to 16.5%, and nitrogen marginally increased from 4.8% to 6.8%. These chemical changes were due to reduced C-C bonds and increased C-O, C=O, C-N, and N-C-O bonds. This proof-of-principle investigation was consolidated by Striesow's in vitro experiment in which collected human forehead lipids were treated using an argon plasma jet (38). The direct-infusion high-resolution tandem mass spectroscopy (DI-MS2) and liquid chromatography-tandem mass spectroscopy (RP-LC/MS2) detected minimal CAP-driven oxidation of triacyclglycerols, ceramides, and cholesteryl esters. In functional term, epidermal lipid overlay could be well protected, and moderate CAP treatment would result in limited negative consequence in the dermal tissue. In addition, Schmidt et al. conducted an in vivo study on murine skin and discovered oxidative modification in the relative abundance of lipid classes using reversed-phase liquid chromatography/mass spectroscopy (39). Finally, Kartaschew et al. used infrared and Raman vibrational microspectroscopy to chemically analyse the plasma-induced change in skin components, such as keratin and lanolin which resembles human sebum (40). The authors suggested that the resultant acidic and NO-containing functional groups could be the source of an antibacterial and regenerative environment in the stratum corneum where plasma interacted with. Although the aforementioned investigational hardware, such as various types of mass spectroscopy, have greatly facilitated the study of the effect of CAP on skin chemistry, limitations of the analytical software's ability to identify unexpected oxidized lipids could lead to an underestimation of CAP's impact on skin lipids (38), suggesting a need for software advancement.

3. Cold Atmospheric Plasma

3.1. Characteristics of Cold Atmospheric Plasma:

In physics, plasma is referred to as the fourth state of matter (after the solid, liquid, and gas phases), or ionized gas, depending on its temperature, and it can be divided into hot plasma and cold plasma. Plasma can be distinguished into standard ("thermal") plasma, at 4000–5000 K, and low-temperature ("cold" or "non-thermal") plasma, at 30–50 °C, which generates oxygen and nitrogen free radicals with positive and/or negative ions [28]. Since high temperatures can cause thermal damage to organisms, the medical field must consider a type of plasma at atmospheric pressure with its temperature close to room temperature [29]. To be considered "plasma," a gas must meet certain criteria, including being molecularly neutral (or near-neutral), having a Debye shield (charged particles capable of counteracting an electrostatic field within a Debye), and having a plasma frequency, defined as the natural oscillation frequency that determines particle movement, causing the gas to return to its neutral state [30].The main components of CAP include ions, electrons, metastables, photons, and electromagnetic fields. After a reaction with environmental air, CAP forms a hierarchical group of reactive oxygen and nitrogen species (RONS) that promote increased skin tissue microcirculation, increased monocyte stimulation, increased cell migration, and stimulation of the keratinocytes and fibroblasts primarily involved in wound healing.Plasma can be applied directly

or indirectly. In the former case, cell lines receive plasma discharges in vitro and animal or human tissue in vivo; in the latter case, a plasma-activated solution is used [30]. To improve CAP efficiency, helium (He), argon (Ar), nitrogen (N2), oxygen (O2), artificial air, and two or more mixtures of these gases can be used to generate CAP [31**].** Energy is required to produce and maintain the plasma. Several devices have been developed in the biomedical field that use electrical energy. Some methods used to produce CAP include: dielectric barrier discharge (DBD), atmospheric-pressure plasma jets (APPJ), plasma needles, and plasma pencils [32].

3.2. Wound Area Reduction

Wound healing is a complex process involving four distinct phases: hemostasis, inflammation, skin proliferation, and remodeling [33]. Wounds can typically be classified as acute and chronic wounds. Acute wounds include abrasions, scalds, burns, or postoperative incisions; chronic wounds do not heal in an orderly manner, frequently remaining in the inflammatory phase for too long and sometimes being complicated by systemic diseases, age, and repeated trauma, such as diabetic ulcers, venous ulcers, arterial ulcers, and pressure sores [34]. CAP may promote wound healing through its antiseptic effects, stimulating the proliferation and migration of skin cells by activating or inhibiting integrin receptors on the cell surface or through its pro-angiogenic effect [35]. It also appears to act by triggering the production of nitric oxide (NO), which promotes cell migration and the assembly of endothelial cells into vessel-like structures useful for wound neo-vascularization [36].Treatment with CAP can be adapted according to the different stages of wound healing; argon plasma was found to be better in promoting coagulation, while helium plasma was more effective in healing [37]. Amini et al. [38] demonstrated that treatment with CAP modifies the persistence levels of inflammatory cytokines and growth factors including IL-1, IL-8, TGF-β, TNF-α, and INF-γ, promoting healing through a more rapid initiation of the proliferative phase. In addition, CAP generates reactive oxygen species (ROS) and nitrogen species (RNS), which can increase the synthesis of pro-angiogenic factors, consequently promoting wound healing [39] (Figure 3 and Figure 4).

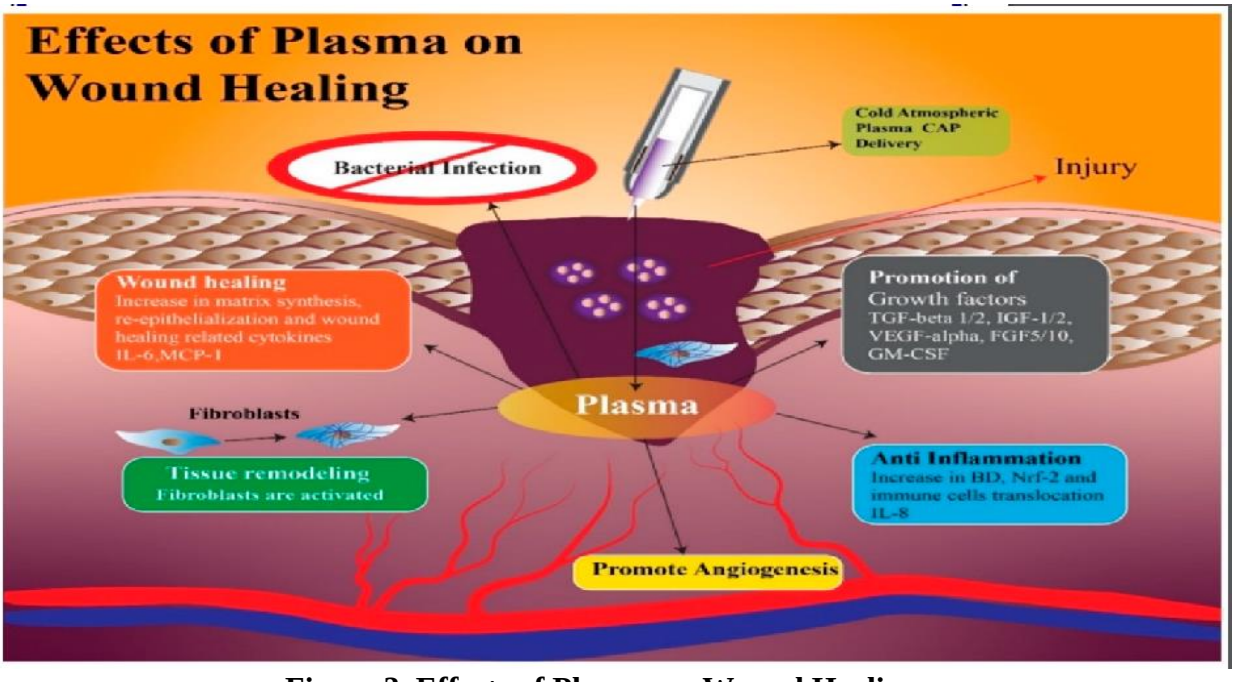

Figure 3. Effects of Plasma on Wound Healing.

With the application of CAP on the wound, there is an increase in growth factors such as Tumor Growth Factor-β 1 and 2; Insulin Growth Factor 1 and 2; Vascular-Endothelial Growth Factor-α; Granulocyte-Macrophage Colony-Stimulating Factor, which facilitate angiogenesis with the anti-inflammatory effect of interleukin-8. Finally, the wound heals with re-epithelialization and synthesis of a new matrix, thanks to the fibroblasts and cytokines, Membrane Cofactor Protein-1 and Interleukin-6.

CAP direct application was used on 27 wounds (19 hard-to-heal wounds and 8 acute wounds), with an average wound area of 15 cm2, for 180 s three times a week in addition to standard therapy. All acute wounds and 68% of the hard-to-heal wounds healed after an average treatment period of 14 weeks; the therapy was only not effective on two hard-to-heal wounds.

Additionally, skin grafting is a unique procedure that removes healthy skin from an area of the body and transplants it into a damaged area. Heinlin et al. [43] enrolled 40 patients to evaluate the impact of cold atmospheric argon plasma on the healing process of the donor site of skin grafts on the upper leg. The investigated wound sites were separated into two distinct areas of the same size and received either CAP indirect therapy or a placebo at random (argon gas) for 2 min. From the second day of treatment, positive effects in terms of improved re-epithelialization, reduction in fibrin layers, and blood crusts were observed in the plasma-treated areas, compared with the placebo-treated areas.

In the recipient site, Frescaline et al. found that CAP could improve extracellular matrix (ECM) formation through activation of the canonical TGF-β1 SMAD-dependent pathway [44] (Figure 3).

An experimental study investigating the effects of argon-jet plasma on donor sites of skin grafts in an environment that was not protein-colonized by bacteria found a positive outcome on wound healing, compared with untreated controls [43].

CAP has also been used to treat burn wounds, hypothesizing an increase in angiogenesis as a determinant of healing. In the study by Betancourt-Angeles et al. [45] on the treatment of human burn wounds, a reduction in pain and itching was found after a three-minute helium CAP application; an extra three-minute treatment after 16 h significantly accelerated healing and the development of new tissue. The authors did not analyze molecular pathways during or after CAP administration since this research was a case report.

The ICU patient very often has wounds due to protracted immobility, critical conditions, or comorbidities, which can cause further complications [46]. Nguyen et al. [47] treated wounds (soft tissue skin lesions, burns, pressure ulcers, shingles, contact, and atopic dermatitis) with CAP at all stages in patients admitted to the ICU with severe COVID-19 infections. A total of 70% of patients who underwent irradiation with CAP as a supportive treatment showed complete epithelialization after 14 days; in addition, cessation of exudation and a reduction in wound pain were noted. The authors point out that in the period before they introduced CAP, these wounds took longer to heal.

Considering the effects of CAP on the activation of biological processes to promote the regeneration and proliferation of various cell types, CAP has also been studied in neuronal regeneration; according to some studies, CAP could promote in vitro differentiation of neuronal stem cells and protect against oxidative stress [48,49].

Nervous system trauma and neurodegeneration often result in permanent functional deficits due to the limited regenerative capacity of the brain and spinal cord. It is known that the central nervous system (CNS) has a limited ability to regenerate following an injury or neurodegenerative disease [50], with a major impact on quality of life for patients. Kativar et al. [51] concluded that astrocytes, glial cells that support neurons through protein secretion of and neurotrophic factors, can

respond to properly calibrated nanosecond-pulse dielectric discharge plasma treatment, which can directly promote their growth and improve their ability to enhance neuronal regeneration after an injury. It is interesting to note the importance of the therapeutic dose on responses: low intensities (≤10 mJ) caused no measurable changes, while high intensities (≥90 mJ) generally resulted in widespread cell death. Intermediate intensities (10–50 mJ) elicited a physiological response, resulting in improved cell regeneration (Figure 3 and Figure 5).

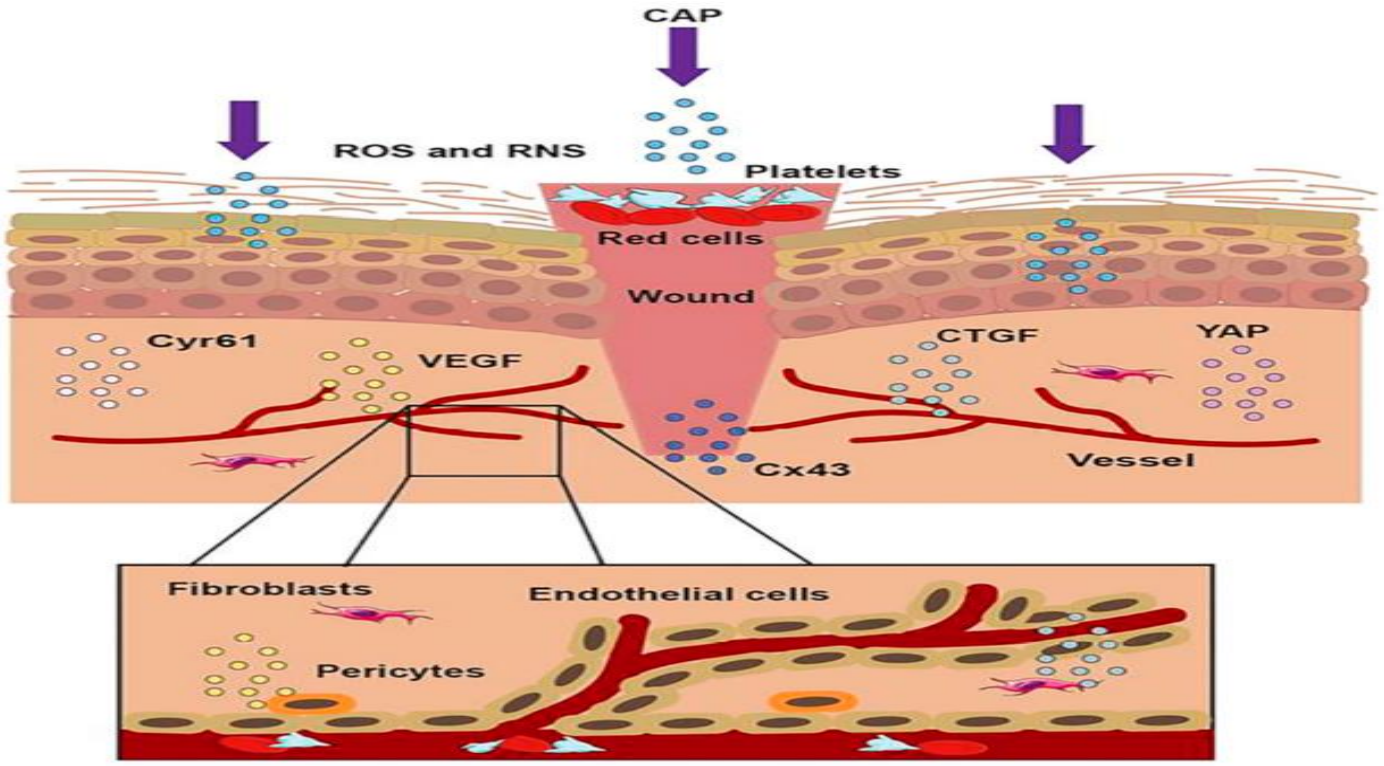

Figure 5. The treatment of CAP on a wound

When the skin was injured, the first step was to form a blood scab to protect the wound. CAP could accomplish wound healing through short-lived and long-lived ROS and RNS. CAP could promote the formation of new blood vessels, strengthen the release of Connective Tissue Growth Factor (CTGF) and Vascular Endothelial Growth Factor (VEGF), activate the Yes-Associated Protein (YAP) pathway, and upregulate the expression of Connexin 43 (Cx43) and Cysteine-rich angiogenic inducer 61 (Cyr61).

3.3. Bacterial Load Reduction:

CAP's bactericidal activity has received 20 years of attention and investigation [50]. The potential uses of CAP for infection control in clinical settings have grown significantly during the last two decades. The ability of CAP to effectively eradicate bacterial biofilms has been demonstrated by several bacterial studies [51,52,53,54]. This is what inspired the concept of utilizing CAP to lower the bacterial wound burden and so improve healing [55]. Early published studies employed jet plasma using argon as the carrier gas. In 2010, a prospective study used argon plasma treatment for 5 min via a CAP device called MicroPlaSter alpha, which resulted in a very significant reduction in bacterial load, compared to standard wound care alone [55]. In fact, after the application of CAP, a mean reduction of 1.10 log10 was observed in the intervention group and a reduction of 0.41 log10 in the control group. Although the difference in the mean reduction between the two arms of the study is significant, an intervention that achieves a bacterial reduction of 1 log10 would hardly be considered

relevant as “bactericidal” or “antimicrobial.” In fact, general agreement indicates that the effective bactericidal action of a device refers to a reduction of at least 3 log10 in the number of viable bacterial cells tested [56,57]. A following study used a second-generation device known as the MicroPlaSter beta, which has a flexible four-joint therapy arm which enables treatment of difficult-to-reach areas. A patient with Hailey-Hailey illness (also known as benign chronic pemphigus) and secondary infections with Candida albicans and Proteus mirabilis saw rapid clinical recovery. Another study [69,74.75.76] clearly demonstrates that a short CAP treatment of 2 min is sufficient to achieve significant reduction in the bacterial load on chronic infected wounds in vivo. Efficacy and tolerability were demonstrated in both generations of devices (MicroPlaSter alpha and MicroPlaSter beta). These studies treated venous, arterial, diabetic, and traumatic ulcers, and reduced bacterial infection was observed regardless of bacterial type.

Maisch et al. [54] reported the efficacy of CAP treatment in decolonizing methicillin-resistant Staphylococcus aureus (MRSA) and Escherichia coli without causing any cellular damage.

Subsequent study results suggest that CAP could be effective for different types of bacteria, including gram-positive and gram-negative bacteria, anaerobes, aerobes, or facultative anaerobes [59]. However, some studies have found resistance of some bacteria to CAP [60,61]. The response of bacteria to CAPs is species-dependent, and gram-positive bacteria have shown greater resistance, indicating the importance of cell wall thickness for the CAP-mediated inactivation time [62]. CAP treatment has been shown to be effective in inhibiting beta-hemolytic streptococci and S. aureus [63], gram-positive aerobic cocci mainly responsible for acute bacterial skin infections [64].

Infection in burn wounds is the most serious consequence of this type of trauma due to the delay in wound recovery and healing; it can also cause bacteremia, sepsis, and multi-organ dysfunction syndromes in the most severe cases, resulting in increased mortality, disease exacerbation, and increased health care burdens [6].

A case report describes the state of a man who suffered two lower-extremity wounds from second-degree burns produced by boiling oil, one with a damaged area of 15 cm2 and the other with a damaged area of 79 cm2. The wounds were inflamed, and the patient experienced severe pain. Three hours after the first treatment with CAP, it was repeated on both wounds for three minutes each. The patient, 16 h after the application of the second treatment, reported no discomfort, whereas a process of re-epithelialization of both wounds and an absence of bacterial infection were observed [45].

Furthermore, CAP displays antibacterial activity via electrostatic stress [65,66]; Das et al.’s [67,74,75] findings suggest that the oxidative stress induced by reactive oxygen and nitrogen species could play a key role in bacterial inactivation.

In 2021, Abbasi et al. [68] examined the effect of CAP on P. aeruginosa isolated from burn infections in vitro and in vivo. The research results showed no bacterial growth, as well as wound healing in mice. The study concluded that CAP decreased the expression of the alp gene that is one of the virulence factors of P. aeruginosa (Figure 6).

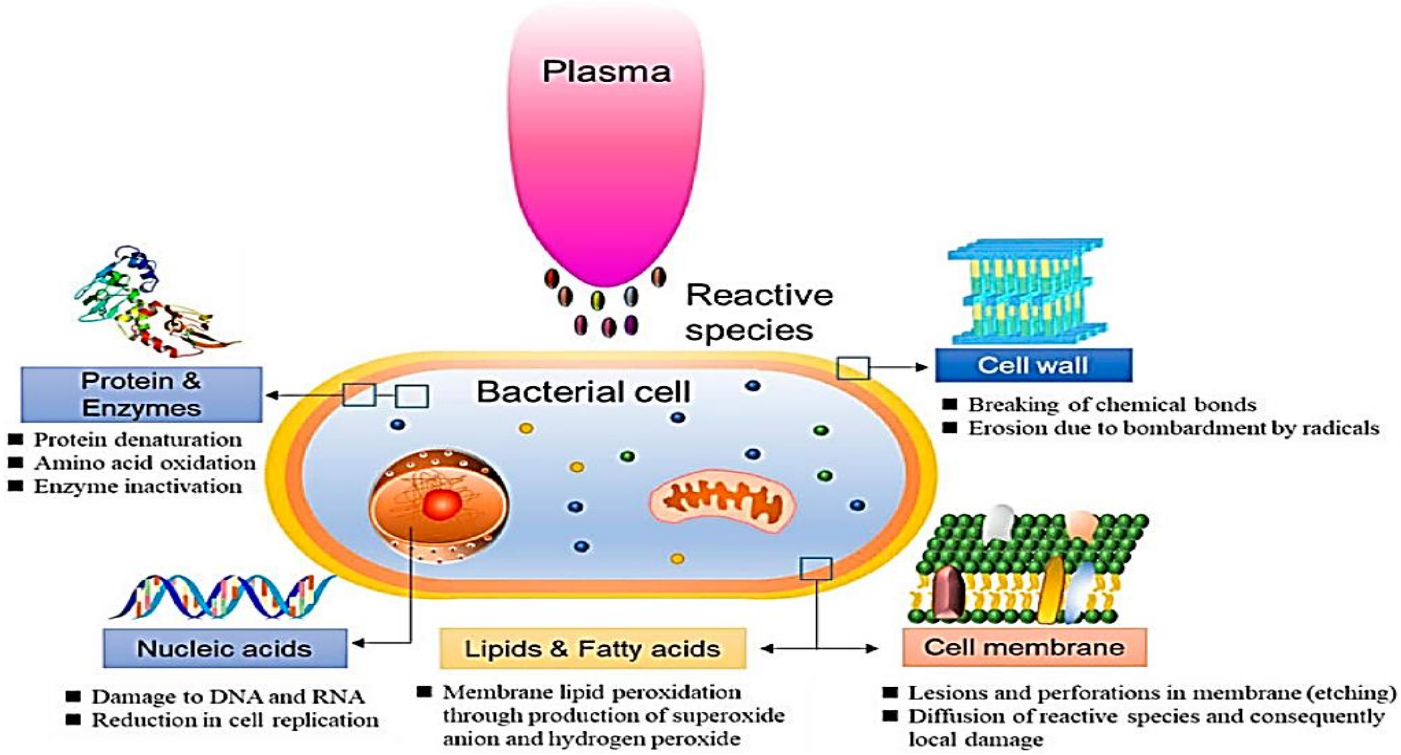

Figure 6. Schematic representation of bacterial reduction induced by CAP

Some studies have described a hybrid treatment for wound healing: CAP therapy combined with antibiotic treatment.

Nguyen et al. [47] applied cold plasma in treating patients with severe COVID-19 who had skin injuries such as burns, pressure ulcers, shingles, and contact or atopic dermatitis. Before the plasma application, all the skin injuries were treated with antibiotics and albumin infusions. After 14 days of cold plasma irradiation, 14/20 patients had complete epithelialization. Previously, when cold plasma irradiation was not applied, these lesions took longer to epithelialize or ulcerated even further, with more exudation despite the use of antibiotics.

In a randomized clinical trial, Stratmann et al. [69,74,76] applied CAP therapy on patients with diabetic foot ulcers. All wounds were treated with systemic antibiotics during the study and were Wagner Armstrong Grade 1B or 2B. Eligible wounds were randomized to receive either a placebo or CAP. In this randomized clinical trial, CAP therapy emerged as an efficient treatment in terms of wound surface reduction and wound closure time.

we tried to summarize the interactions between cold atmospheric plasmas and the skin, based on the current knowledge of their own properties and characteristics, highlighting the already known and some other possible effects of these sources on the integumentary system biology (Fig. 7). We described how the multiple physical parameters involved in cold plasma can individually or together impact the cutaneous microenvironment and skin cell activities. The coaction of all these effects tends towards a beneficial role of CAPs on skin biology and brings relevant arguments in favor of the use of cold plasma for restoring skin functional barrier and thus improving skin health and appearance. [70,73.77].

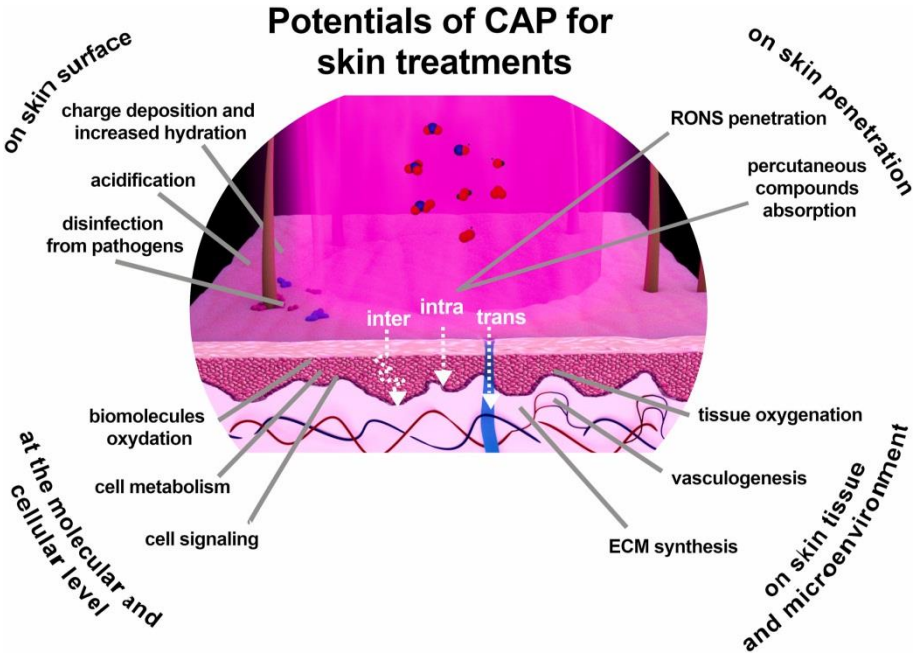

Fig. 7. Potentials of CAP in skin biology.

CAP performs its activity at various levels of the skin. At a superficial level it promotes the hydration, acidification and decontamination of the stratum corneum. CAP-generated RONS can penetrate inside the skin via the intercellular way (inter), the intracellular way (intra) or via the transappendageal way (trans). By loosening the cutaneous barrier, CAP also promotes the absorption of other molecules such as drugs. At a molecular level, once penetrated into the skin, RONS can have a direct effect on skin biomolecule oxidation or activate cell metabolism and signaling. At the tissue level, CAP treatment lead to an increase in skin oxygenation, stimulates the vasculogenesis and the ECM remodeling or de-novo synthesis.

Conclusion

CAP is effective in many areas of medicine, without significant negative effects on healthy cells. However, its use might have potentially serious side effects and should, thus, be used under expert supervision and with adequate dosages. In this context, this procedure may be standardized and implemented to clinical practice in the future to achieve favorable outcomes in a safe and effective manner. Furthermore, the efficacy of CAP in the treatment of wounds, particularly pressure sores, will allow for a reduction in nosocomial infections, resulting in an improvement in the quality of hospital life and, as a result, a significant reduction in morbidity and mortality associated with HCAIs and bedsores, which also covers the legal implications of this type of therapy. As a result, the focus on the use of CAP and the findings of numerous studies may drive the advancement of technical research, allowing the creation of ever-smaller devices capable of delivering plasma to inside structures.

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