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REVIEW

Clinical and Biological Principles of Cold Atmospheric Plasma Application in Skin Cancer

Jesús Gay-Mimbrera · Maria Carmen García · Beatriz Isla-Tejera · Antonio Rodero-Serrano · Antonio Vélez García-Nieto · Juan Ruano

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ABSTRACT

Plasma-based electrosurgical devices have long been employed for tissue coagulation, cutting, desiccation, and cauterizing. Despite their clinical benefits, these technologies involve tissue heating and their effects are primarily heat-mediated. Recently, there have been significant developments in cold atmospheric pressure plasma (CAP) science and engineering. New sources of CAP with well-controlled temperatures below 40 °C have been designed, permitting safe plasma application on animal and human bodies. In the last decade, a new innovative field, often referred to as *plasma medicine*, which combines plasma physics, life science, and clinical medicine has emerged. This field aims to exploit effects of mild plasma by controlling the interactions between plasma components (and other secondary species that can be formed from these components) with specific structural elements and functionalities of living cells. Recent studies showed that CAP can exert beneficial effects when applied selectively in certain pathologies with minimal toxicity to normal tissues. The rapid increase in new investigations and development of various devices for CAP application suggest early adoption of cold plasma as a new tool in the biomedical field. This review explores the latest major achievements in the field, focusing on the biological effects, mechanisms of action, and clinical evidence of CAP applications in areas such as skin disinfection, tissue regeneration, chronic wounds, and cancer

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J. Gay-Mimbrera · B. Isla-Tejera · A. V. García-Nieto · J. Ruano (✉)
Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Hospital Universitario Reina Sofía, University of Córdoba, Córdoba, Spain
e-mail: juanruanoruiz@mac.com

M. C. García
Department of Applied Physics, University of Córdoba, Córdoba, Spain

B. Isla-Tejera
Department of Pharmacy, Hospital Universitario Reina Sofía, Córdoba, Spain

A. Rodero-Serrano
Department of Physics, School of Engineering Science of Belmez, University of Córdoba, Córdoba, Spain

A. V. García-Nieto · J. Ruano
Department of Dermatology, Hospital Universitario Reina Sofía, Córdoba, Spain

treatment. This information may serve as a foundation for the design of future clinical trials to assess the efficacy and safety of CAP as an adjuvant therapy for skin cancer.

Keywords: Apoptosis; Cold atmospheric plasma; Dermatology; Keratinocytes; Melanoma; Non-melanoma skin cancer; Oncology

CHARACTERISTICS OF COLD ATMOSPHERIC PLASMA

Plasma is an ionized gas that is composed of ions, electrons, photons, and neutrals (radicals as well as excited atoms and molecules). All are active species capable of inducing different physical phenomena and chemical reactions. There are many examples of plasma in nature, such as plasmas that are generated in stars and the polar aurora. Plasmas can also be created in the laboratory setting; here, plasmas are maintained by applying an external source of energy, generally an electromagnetic field, to a gas. Plasma technology has gained much interest in recent years owing to its various applications in fields such as microelectronics, waste elimination, lighting, and textile. Several nonthermal plasma sources have been recently developed. These plasma sources can be well controlled and open to the air, allowing for the maintenance of CAPs with temperatures below 40 °C. These developments have encouraged therapeutic application of CAPs and the emergence of plasma medicine technology.

Nonetheless, plasma application on the human body for medical purposes has a significant history. In the mid-nineteenth century, electrotherapy was introduced as a therapeutic approach, and spark and flash discharges were employed to treat several diseases. Later, electrosurgical techniques based on the use of plasmas were developed. In electrosurgery, selective coagulation or tissue cutting is achieved by tissue heating which results in desiccation of cells, denaturation of proteins, or devitalization of tissues [1]. Argon plasma coagulation (APC) is a well-established method in the field and commonly employed today for tissue coagulation during endoscopy (in gastroenterology, general and visceral surgery, urology, or gynecology) [2]. APC is a monopolar technique introduced in the 1970s, in which electrical energy is transferred to target tissue as current by means of argon plasma. This technique competes with traditional laser ablation. Comparative studies have shown that APC is more effective for tissue destruction owing to its superior energy concentration. Furthermore, PlasmaJet®, another electrosurgical technique, possesses activity that is mostly mediated by thermal (thus destructive) interaction with living tissues. It consists of a bipolar electrode system with low-flow argon as the process gas. This technique is typically employed to cut or coagulate tissues in a well-defined and localized manner. These direct plasma applications on living tissues in electrosurgery are based on extreme interactions of plasma with cells or tissue, which may lead to cellular destruction and local “sealing” of tissue.

In the last decade, with the development of CAPs, modern plasma medicine has emerged. This branch of medicine aims to exploit the effects of mild plasma by utilizing the distinct interaction of plasma components (and other secondary species than can be formed from these) with specific structural elements as well as functionalities of living cells [1]. These interactions may lead to either stimulation or inhibition of cellular function; thus, this technique may be employed for various

therapeutic purposes [3]. While most clinical studies have been conducted in the area of dermatology, interests in CAP technology have also emerged from other disciplines, such as oncology, surgery, otolaryngology, gastroenterology, and odontology [4, 5].

Sources of Cold Atmospheric Plasma

New CAP sources used in plasma medicine can be classified into three types [6]:

1. *Direct plasma sources* These plasmas use the human body (such as the skin, internal tissues, etc.) as an electrode; thus, the current produced by plasmas has to pass through the body. The most utilized technology in this category is the dielectric barrier discharge (DBD) plasma source.
2. *Indirect plasma sources* These plasmas are generated between two electrodes. Active species that are created by the plasmas are subsequently transported to target application areas. Several devices are available, ranging from very narrow plasma needles or jets to larger plasma torches such as the kINPen® MED, Atmospheric Pressure MicroPlasma Jet (APMPJ), and MicroPlaSter® α and β.
3. *Hybrid plasma sources* These plasmas combine the benefits of the two aforementioned plasma source types (e.g., using the plasma production technique of direct plasma sources and the essentially current-free property of indirect plasma sources). This is achieved by introducing a grounded wire mesh electrode, which has a significantly smaller electrical resistance than that of the tissue; thus, in principle, all current can pass through the wire mesh. The MiniFlatPlaSter is an example of a hybrid plasma source.

Nevertheless, plasmas can also be generated by discharges in air, noble gases, or any desired mixture. They can be excited by various means, including radio frequency, microwave frequencies, and high voltage AC or DC, in continuous or pulsed mode in order to produce a chemical cocktail of active species for biomedical applications. Table 1 summarizes the properties of various types of plasma reactors that have been employed for dermatological purposes. In cold atmospheric pressure plasma sources, the main reactive components are reactive neutral species (including free radicals and few ground state molecules such as peroxides and ozone) and UV radiation. Because CAP sources operate at atmospheric pressure while in contact with air, they generate copious quantities of reactive oxygen species (ROS) and reactive nitrogen species (RNS), collectively referred to as RONS. A list of RONS was provided in a recent review, which also highlighted the role of these species in redox biology and their implications for therapeutic applications of plasma [7]. RONS can strongly influence cellular biochemistry and are known to be important in animal and plant immune systems, supporting the notion that they are indeed key mediators in CAP therapeutics.

Here, we review the latest evidence of the biological effects, mechanisms of action, and clinical benefits of CAP application in dermatology. This review also highlights the potential application of CAP as co-adjuvant therapy for the treatment of skin tumor. Analyses described in this review article were based on previously conducted studies. No studies involving human subjects or animals were performed for the publication of this review.

Table 1 Summary of plasma sources used in dermatology

Type of plasma source	Plasma source	Device	Gas	Frequency	Flow	Power/ discharge voltage	Treatment
Direct plasma source	DBD	PlasmaDerm®, CINOGY GmbH	Ar	DC Pulsed 100–400 Hz	8 slm	0.17–0.24 W/ 14 kV	Melanoma [36] Chronic ulcer [42] Skin moisture [47]
		Custom design	$Ar + O_2$	DC Pulsed 60 Hz	No flow	0.9 W/20 kV	Keratinocytes effect [15]
		Custom design	Air	DC Pulsed 255–518 Hz	No flow	0.2–0.4 W/ 5–6 kV	Wound healing [49]
		Custom design (point to plane arrangement)	Air	100 kHz Pulsed 300 Hz	No flow	14 kV	Skin treatment (physical model) [50]
		DBD, INP Greifswald	Air	31 kHz	No flow	0.4–1.6 W/ 13 kV	Study of skin irritation [51]
		DBD plasma strip	Air	6.6 kHz	No flow	0.7 W/3.5 kV	Infected wound [52]
		Direct DBD-Bioplasma cell modulation	Air	15–20 kHz Pulsed 10–110 Hz	No flow	40 W/ 6–7 kV	Acne and aesthetic treatment [53]
		Surface DBD	Air/Ar	20–21 kHz	No flow/ 0.5 slm	0.14–0.3 W/ 3.5–10 kV	Wound healing [54]
		Volumen DBD	Ar	33 kHz	0.5 slm	6 W/ 9–10 kV	Wound healing [54]
Indirect plasma source	Jets	kINPen® MED, neoplas tools GmbH	Ar	1–1.5 MHz	3–8 slm	1–6 kV	Skin decontamination [55] Psoriasis [43] Melanoma [50] Skin moisture [31] Plasma effect in stratum corneum [56] Wound healing [4, 18, 57, 58]

Table 1 continued

Type of plasma source	Plasma source	Device	Gas	Frequency	Flow	Power/ discharge voltage	Treatment
		Custom design	Ar/ He	10 kHz	2 slm	8/10 kV	Human mesenchymal stromal cells [30]
		Custom design	He	230–270 kHz	2 slm	1.1–1.8 kV	Melanoma [35, 57]
		APMPJ	He	60 kHz	1 slm	5–5.5 kV	Endoscopic sterilization [4]
		Custom design	He	RF 25 kHz Pulsed 400 Hz	16.5 slm	25 kV	Wounds healing [58]
		HF plasma jet	Ar	1.82 MHz Continuous/ pulsed 10 kHz	2 slm	2–3 W/ 0.4–0.6 W	Study of plasma irritation [51]
		RF APPJ	Ar	13.56 MHz	1.5 slm	1.7 W	Antibacterial [59] Infected wound [51]
	Torches	MicroPlaSter®, terraplasma GmbH	Ar	2.45 GHz	2.2–4 slm	86–110 W	Wound healing [9, 24, 40, 41] Keratinocytes effect [19] Pruritus [45]
Hybrid plasma source	SMD technology	Custom design	Air	12.5 kHz	No flow	0.5 W m^{-2}/ 18 kV	Nosocomial infections [60]
		MiniFlatPlaSter	Air	Pulsed 6.75 kHz	No flow	7 kV	In vivo skin disinfection (bacteria) [61] Ex vivo nasal and pharyngeal mucosa [48] Melanoma [13]

SMD surface micro discharge, *RF* radio frequency, *HF* high frequency, *APMPJ* atmospheric pressure microplasma jet, *APPJ* atmospheric pressure plasma jet, *DBD* dielectric barrier discharge, *Ar* argon, *slm* standard liter per minute

BIOLOGICAL EFFECTS AND MECHANISMS OF ACTION OF COLD ATMOSPHERIC PLASMA

CAP is a gas composed of multiple chemically active species. It induces physical and chemical changes on biological surfaces upon application. In recent years, several studies have demonstrated biological effects of these changes. Elucidation of the key mechanism behind the effects of CAP in cancerous cells will be crucial to determine the optimal dose of CAP for clinical use.

Oxidative Stress

The level of intracellular ROS and RNS (RONS) is tightly regulated by small antioxidant molecules and scavenging enzymes. At low concentrations, RONS are involved in various biological processes such as bacterial destruction by macrophages and endothelial nitric oxide-mediated vasodilatation. However, when their levels exceed the capacity of the redox balance control system, a state referred to as oxidative stress, they can be cytotoxic and cause cell death. Oxidative stress is involved in the development of various diseases such as psoriasis, chronic ulcers, and cancer.

Cancer cells display weaker antioxidant mechanisms when compared to normal cells. This property can facilitate selective attack of cancer cells by CAP mediated by the extracellular RONS, resulting in severe oxidative damage and cell death. Schmidt et al. observed that alterations in redox state due to CAP treatment caused changes in cellular morphology and mobility, but not cell viability [8]. The authors also found that oxidative stress induced by CAP can modify the expression of nearly 3000 genes encoding structural proteins and inflammatory mediators, such as growth factors and cytokines.

Gene Expression and Epigenetic Changes

Numerous studies have assessed the effects of CAP on gene expression and epigenetics in several cells lines. Application of CAP for 2 min with the MicroPlaSter β® device on a fibroblast culture and in a wound healing mouse model increased the expression of type I collagen and genes encoding proteins involved in wound healing processes (interleukin 6 [IL-6], IL-8, chemokine [C–C motif] ligand 2 [CCL2], transforming growth factor beta 1 [TGF-β1], TGF-β2, CD40 ligand, chemokine [C–X–C motif] ligand 1 [CXCL1], interleukin 1 receptor antagonist [IL-1RA], and plasminogen activator inhibitor-1 [PAI-1]) without affecting cellular migration, proliferation, and apoptosis [9]. Zhong et al. demonstrated the downregulation of IL-12 and upregulation of IL-1β, IL-6, IL-8, IL-10, tumor necrosis factor α (TNFα), interferon gamma (IFNγ), and vascular endothelial growth factor (VEGF) mRNAs when CAP was applied to keratinocyte cultures [10]. Park et al. demonstrated for the first time changes in DNA methylation pattern following CAP application in a breast cancer cell line expressing the estrogen receptor (MCF-7) and one that does not express it (MDA-MB-231). Epigenetic modifications were more extensive in MCF-7 cells, affecting the promoter region of genes related to "cell mobility", "connective tissue function and development", "motility development", "cell–cell communication and cell–cell interaction", and "cell survival and cell death" [11].

Mitochondria, Cell Cycle, and Apoptosis

Apoptosis is a type of programmed cell death, and mitochondria act as the major regulator of apoptosis. Various intracellular and extracellular signals induced by CAP-mediated oxidative stress converge in mitochondria, increasing their transmembrane potential and promoting the release of pro-apoptotic factors including cytochrome c. This process is regulated by the Bcl-2 protein family and ultimately leads to the activation of the caspase cascade [12]. Arndt et al. showed that when CAP was applied for 2 min to a melanoma cell line, pro-apoptotic changes such as Rad17 and tumor suppressor p53 phosphorylations, cytochrome c release, and caspase-3 activation were initiated [13].

The cell cycle is a series of events leading to cell replacement in tissues. RONS produced following high dose application of CAP can alter the cell cycle, which typically leads to apoptosis. However, lower doses of CAP can also inhibit cell proliferation by inducing cell senescence, especially when most cells in the tissue are in the proliferative phase, as observed in most tumors [13]. Typically, normal tissues differ from tumor in the proportion of cells in each cell cycle phase at a given time. In fact, this could be the biological mechanism behind the high selectivity of CAP to induce apoptosis of these cells while preserving viability of non-tumor cells. Yan et al. demonstrated that CAP increased the percentage of apoptotic tumor cells by blocking the cell cycle at the G2/M checkpoint, and this effect was mediated by reduced intracellular cyclin B1 and cyclin-dependent kinase 1 (Cdc2), increased p53 and cyclin-dependent kinase inhibitor 1 (p21), and increased Bcl-2-like protein 4 (Bax)/B cell lymphoma 2 (Bcl-2) ratio [14]. However, it is important to mention that the viability of non-tumor cells can also be altered if cells are exposed to CAP for a longer period of time [15].

EFFECTS OF COLD ATMOSPHERIC PLASMA ON NORMAL SKIN CELLS

In a laboratory setting, several studies have been performed to determine the effect of CAP applied to cells that are part of the epidermal (i.e., keratinocytes and melanocytes) or dermal (fibroblasts) cytoarchitecture. In these studies, dose-dependent effects of CAP on cells were observed. CAP application for less than 2 min on keratinocytes and fibroblasts was not associated with increased cell toxicity or apoptosis. However, lower or higher doses may stimulate or inhibit cell migration (fibroblasts) and proliferation (fibroblasts and keratinocytes), respectively. Most of these studies utilized normal melanocytes as a control for melanoma cells. The latest evidence of the effects of CAP on skin cells is summarized in this review.

The antiproliferative effects of CAP have been associated with increased numbers of keratinocytes in the G2/M1 phase [16]. Wende et al. evaluated 40-s CAP application on an in vitro model of wound healing based on culture of human keratinocytes colonized by *Staphylococcus epidermidis* [17]. Bacterial load reduction and closure of artificial wound were improved following CAP application when compared to the control. Hasse et al. investigated the ex vivo effects of CAP applied to healthy human skin samples for a longer period of time. In this study, while epidermis integrity and expression pattern of various keratins remained unchanged, basal proliferation of keratinocytes was found to be increased after 1–3 min of CAP exposure. Apoptosis was induced only when CAP was applied for 3–5 min [18]. This proliferative effect achieved using short exposure time may be

beneficial to expedite healing processes. Other studies have demonstrated increased expression of IL-8, TGB-1β/TGB-β2, and β-defensin mRNA 24–48 h following keratinocytes exposure to CAP for 2 min, with no observable modification of cell proliferation, migration, or apoptosis [19].

Wound healing is a complex and dynamic biological process that requires the sequential coordination of cells, cytokines, chemokines, and proteins of the renin–angiotensin system. The time when resident fibroblasts achieve the capacity to produce growth factors and generate a collagen network is a critical point in the tissue restoration process [20]. Shashurin et al. observed that fibroblast adhesion and migration were halved following 5-min CAP application, which appeared concurrently with the downregulation of α and β integrins (10% and 22%, respectively) [21]. A subsequent study, in which CAP was applied on a human skin-derived fibroblast culture for less than 1 min, showed absence of cell proliferation and apoptosis changes [22]. However, opposing effects were observed in other studies. Tipa and Kroesen applied CAP on a cell culture-based wound model for 5–15 s and found that fibroblasts were able to cover the artificial wound more rapidly without any observable cytotoxic effects [23]. The observed increase in the proliferative and migration capacities of fibroblasts may be linked to peroxisome proliferator-activated receptor gamma (PPARγ) activation mediated by elevated intracellular ROS [19].

STANDARDIZATION OF PROCEDURES AND SAFETY ASSESSMENT

Several factors can influence the interpretation of the effects of CAP on cells and tissues, making it challenging to compare results obtained by different researchers. A standardized system of procedures related to plasma sources, devices, and treatment doses used in each study is necessary. Currently, only the DIN General Requirements of Plasma Sources in Medicine (DIN SPEC 91315, 2014), which was presented at the 5th International Conference on Plasma Medicine (ICPM5), has been published.

While most studies conducted in human subjects have described the short-term safety profile of the plasma device, there is currently no consensus on which strategy should be used to address this issue. In several studies, a tissue tolerable plasma (TTP) was determined. For example, Isbary et al. evaluated the tolerability and safety of CAP applied using the FlatPlaSter 2.0 and MiniFlatPlaSter devices through histology, electron microscopy, and DNA damage evaluations [24]. Ma et al. determined that the intracellular mechanisms were the most effective in protecting cells from oxidative stress induced by plasma by reducing cell death [25]. Lademann et al. focused on assessing the effects of UV radiation and temperature on the skin following CAP applications in several patients and healthy volunteers. They showed that UV radiation emitted by CAP was an order of magnitude lower than the minimal erythemal dose (the minimum dose needed to produce sunburn in the skin in vivo) and no thermal damage was observed in the CAP-treated areas [26]. Wende et al. recently used standardized procedures to evaluate the mutagenic potential of kIN-Pen® MED plasma in the clinic [27]. They demonstrated that RONS generated by the plasma were not able to interact directly with DNA or were found in low concentrations, which should allow for DNA damage repair by cellular mechanisms. Thus, plasma was determined to be non-genotoxic to human

cells in vitro. Lastly, it should be highlighted that despite these attempts, in vivo studies evaluating potential long-term side effects of CAP have yet to be conducted.

ANTITUMOR EFFECTS OF CAPS AND THEIR POTENTIAL APPLICATION IN DERMATOLOGY

CAP has shown a significant anticancer capacity over a wide range of cancer types. Several studies have found that tumor cells are more sensitive to CAP compared to normal cells; thus, this technology should be considered for an ideal cancer treatment.

As described previously, CAP can selectively induce apoptosis of tumor cells [28]. This feature supports CAP as a new therapeutic tool that complements the clinical benefits obtained with conventional treatments, as the latter may result in the damage of surrounding healthy tissues and are associated with greater treatment costs and/or risk of adverse effects. In contrast to chemotherapy and radiotherapy, the most attractive feature of CAP is its selective capacity for killing cancer cells. To date, several studies have demonstrated the benefit of CAP jet application on culture of cells obtained from human tumors or in immortal cell lines and animal models (Table 2). The selectivity of CAP was not only observed in cancer cells but also in various cancer cell lines. The killing capacity of CAP is dose-dependent and inversely proportional to the growth speed of cancer cells. Different studies have examined the effects of CAP on cell adhesion, migration, and invasive capacities. CAP can decrease cell adhesion without causing necrosis. In fact, CAP was able to induce detachment of certain cells through the action of ROS on the outer cell membrane without necessarily causing any intracellular changes [29]. This effect appeared to be reversible; thus, it can serve as the basis of future CAP applications in tumor microsurgery.

Data related to the percentage or timing of the improvement achieved after CAP treatment vary widely among studies. There are many methodological differences (type of CAP, exposure time, and distance to cells) that make it difficult to compare the results (i.e., cell apoptosis after CAP application varies from 20% to 40% of melanoma cells and <10% of melanocytes) [13]. Related to the time needed to achieve the improvement, most of the reviewed studies were performed after aplying CAP for 1–180 s on cell cultures (Table 2). Short-time effects were assessed after 1–3 days in most of the cases; longer periods are needed to explore chronic effects, but in that case is not possible to use cell cultures because of confluency of cells; thus, animal models should be used for that purpose.

In most studies, CAP was applied directly to cells or tissues. However, over the past 4 years, CAP-irradiated media have also been found to effectively kill cancer cells. These media were used on mesenchymal stroma and LP-1 myeloma cell lines, and the majority of the observed effects were mediated by H_2O_2 and O_2 species [30]. Yan et al. recently determined H_2O_2 as the main reactive species and cysteine as the central target molecule of CAP-irradiated media used on glioblastoma and breast cancer cells [31]. Plasma-activated water (PAW), an example of a CAP-irradiated media, is a promising anticancer therapeutic that has several advantages over the direct CAP application. These advantages should be accounted for if PAW implementation in the clinic is considered. PAW can be stored in a refrigerator for 1 week without losing its anticancer properties [32]. This feature will

Table 2 Biological effects of cold plasma in preclinical models of various cancers

Non-skin tumors	Plasma	Exposure time	Model	Explored features
Lymphoma [62]	DBD plasma	Various, from 30 to 480 s	Human monocytic lymphoma cell line (U937)	Metabolic activity, cell viability, and apoptosis
Breast cancer [63]	Custom design	30, 60, and 120 s	Human metastatic cell line (MDA-MB-231)	Cell proliferation and migration
Ovarian cancer [64]	NEAPP	Various, from 2 to 600 s	Ovarian cancer cell lines (SKOV3 and HRA)	Cell proliferation and apoptosis
Colorectal cancer [65]	Torch with spray	1 s	Colorectal cancer cell lines	Migration and invasion
Lung cancer [66]	Plasma plume	10 s	Human lung adenocarcinoma cell line (A549)	DNA damage and cell viability
Liver cancer [67]	Microsize jet-type plasma	2 min	Human liver cancer cell line (SK-HEP-1)	Cell adhesion
Lung carcinoma [68]	Custom design (CU)	20 s	TC-1 mouse lung carcinoma cells	Apoptosis
Pancreatic cancer [69]	Plasma jet kINPen 09	5, 10, and 20 s	Human pancreatic cancer cell line (Colo-357 and PaTu8988T)/murine cell line (6606PDA)	Cell viability and apoptosis
Non-cutaneous squamous cell carcinoma [70]	Custom design (GWU)	10, 30, and 45 s	Head and neck squamous cell carcinoma cell lines	Cell viability and colony formation
Prostate cancer [71]	Custom design	–	Human and cell lines	Cell viability, gene expression, and apoptosis
Prostate cancer [72]	μ-APPJ	Various, from 2 min to 20 min	PC-3 prostate cancer cells	Cell viability, protein expression, and nitric oxide quantitation
Prostate cancer [73]	kIN-Pen Med	10 s	Human epithelial PC cell lines (LNCaP and PC-3)	Cell proliferation and apoptosis
Glioma [74]	DBD plasma	–	Human glioma cell line (U373MG)	Cell viability
Glioblastoma [75]	DBD plasma	20 s/day × 3 days	U87-Luc glioma tumor on athymic BALB/c nude and C57bl6 mice	Temperature and antitumor effects
Glioblastoma [76]	SMD plasma	30, 60, and 120 s	Human glioblastoma cancer cell lines	Cell viability, DNA damage, and cell cycle

Table 2 continued

Non-skin tumors	Plasma	Exposure time	Model	Explored features
Glioblastoma [77, 78]	Custom design (GWU)	Various, from 60 to 180 s	Human glioblastoma cancer cell line (U87)	Cell viability, cell cycle, and apoptosis
Glioblastoma [79]	DBD plasma	30, 60, 90, and 180 s	Glioma cell lines (U87, U373, A172), human normal astrocytes E6/E7, and HUVEC	Cell viability, cell cycle, and apoptosis
Neuroblastoma [80]	Helium-based plasma	0, 30, 60, and 120 s	Neuro2a cells murine neuroblastoma	Metabolic activity and apoptosis

HUVEC human umbilical vein endothelial cells, *μ-APPJ* micro-scaled atmospheric pressure plasma jet, *GWU* George Washington University, *NEAPP* non-equilibrium atmospheric pressure plasma, *DBD* dielectric barrier discharge, *CU* Clemson University, *SMD* surface micro discharge

permit the central production of PAW in hospitals using a single plasma generator. PAW can then be packaged and distributed to various operating rooms as needed for cancer treatments within the same day. Moreover, PAW can be applied topically on tumor surfaces or injected into the tumor [33]. This feature is significant in dermatology because skin tumors are easily accessible using these approaches. However, basic principles to guide PAW application in cancer, specifically to treat skin cancer, remain undefined.

Oxidative stress may play various roles in the pathogenesis of human skin cancers. The most clinically relevant dermatological tumors are the basal cell carcinoma, cutaneous squamous cell carcinoma, and malignant melanoma. Melanoma cells exhibit increased oxidative stress, which could damage surrounding tissue, thereby supporting the progression of metastasis [34]. When CAP was applied on an immortal melanoma cell line for 2 min, apoptosis was induced in cancer cells, but not in non-neoplastic melanocytes [13]. CAP treatment for 1 min did not induce apoptosis, although a prolonged inhibition of cell proliferation was observed, promoting cell senescence. Importantly, this demonstrated the ability of CAP application to remove tumor cells from the proliferative phase of the cell cycle. Ishaq et al. also observed a similar effect by comparing a line of melanoma cells to melanocytes in culture [35]. Elevated intracellular ROS induced the expression of genes involved in cellular apoptosis mediated by TNFα and apoptosis signal-regulating kinase (ASK). When cells were pretreated with *N*-acetylcysteine and an antibody against TNFα, the apoptosis signal was inhibited. Recently, Daeschlein et al. evaluated the antitumor efficacy of CAP administered in conjunction with a bleomycin-based electrochemotherapy in a mouse model of melanoma. The combinational therapy improved mice survival significantly when compared to the electrochemotherapy alone [36].

To date, experimental and/or clinical studies of CAP use in non-melanoma skin cancer (such as basal cell carcinoma and squamous cell carcinoma) have yet to be conducted. In non-melanoma skin cancer, diminished

antioxidant defense caused by chronic UV exposure may contribute to carcinogenesis [34]. A related study, assessing the use of CAP in cultured explants of human low-degree non-cutaneous epidermoid carcinoma of the head and neck, demonstrated that plasma can decrease cell viability and increase DNA fragmentation and cell apoptosis [37].

CLINICAL USE OF CAP IN HUMAN SKIN

Few studies have addressed the effects of CAP in humans, and studies related to skin cancer have not been performed. Most clinical trials evaluated the efficacy and safety of cold plasma application, mainly as adjuvant treatment of chronic cutaneous ulcers. This is due to the frequent bacterial colonization or wound infection in chronic cutaneous ulcers that may affect adequate restitution of tissue structure and function. Furthermore, development of bacterial resistance is unlikely based on CAP mechanism of action [38, 39]. Study design and information obtained on CAP efficacy and safety can serve as a foundation to develop future clinical trials to assess CAP as a treatment option for skin cancer [40–42]. Virtually all clinical studies of CAP have been performed using medical devices designed for topical application (MicroPlaSter® α, MicroPlaSter® β, and PlasmaDerm® VU 2010), following a phase I or II clinical trial design using small numbers of healthy volunteers or patients (from 14 to 70). In all cases, treatment was well tolerated and no significant differences in adverse effect frequency were observed in the CAP group as compared to the controls. Results obtained from other studies on CAP application in human subjects are circumstantial; most were communicated as case reports or small series of cases, and all studies were performed in non-cancerous skin diseases. In most studies, CAP treatment was not more effective than the placebo; however, it was well tolerated with no relevant adverse events [43–45]. Recently, Metelmann et al. published a retrospective review of 12 patients with head and neck non-cutaneous advanced squamous cell carcinoma that were treated with CAP to decontaminate infected cancer ulcerations. When they evaluated anticancer effects, superficial partial remission of tumor was observed in some cases following CAP exposure [46].

CONCLUSION

The recent development of new plasma sources and devices for simple CAP application on the skin paralleled numerous studies performed in vitro, ex vivo, and in human subjects. When applied directly or indirectly on cell cultures or disease models (in vitro or in vivo using animal models), CAP has been shown to reduce cell proliferation, adhesion, and migration, and induce selective apoptosis of neoplastic cells without damaging normal cells. These selective effects may be due to differences in the intracellular oxidative status and cell cycle phase between normal and tumor tissues. Furthermore, excessive levels of oxidative radicals induced by CAP can induce DNA damage and cell cycle exit into senescence, apoptosis, or necrosis. The antitumor capacity of CAP treatment can been regulated by controlling treatment time, gas source composition, gas flow rate, and supply voltage. When CAP is utilized to irradiate a medium or water to obtain WAS, the distance between CAP source and the liquid, and the final volume should be taken into consideration. Despite mounting evidence supporting its use, studies

of CAP in human subjects are still limited. In the majority of these studies, CAP was shown to be well tolerated without any observable short-term adverse effects. Therefore, we suggest further investigation of CAP, including the use of PAW, as potential adjuvant therapies for skin tumors such as basal cell carcinoma, squamous cell carcinoma, and malignant melanoma.

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