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Review Article

Dentistry Section

A Review on Therapeutic Potential of Cold Atmospheric Plasma Therapy in Oral Cancer: Emerging Trends and Amelioration

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ABSTRACT

Oral Squamous Cell Carcinoma (OSCC) is a life-threatening disease. Many patients are in advanced stages at the time of diagnosis, resulting in high mortality, morbidity and clinical problems that make clinical management challenging. Due to advancements in early diagnostic procedures of cancer and its treatment, the number of patients suffering from OSCC has decreased. Still, the number of death due to cancer has not been reduced. In many circumstances, chemotherapy has major side-effects. Cold Atmospheric pressure Plasma (CAP) is a new therapy option that seems to be used mostly as part of a palliative cancer treatment program to provide comfort to these patients. Using a gas that is ionised partially, known as CAP therapy, researchers are now able to treat cancer. The identification of the anticancer properties of CAP, and clinical efficacy, can open up the door for the development of a wide range of synergistic and individualised plasma-enabled therapies. These drugs can potentially enhance current therapies in the direction of safer, more efficient treatment modalities and provide a gentle but efficacious cancer single-agent therapy with a wide therapeutic window and good selectivity.

Keywords: Cold plasma, Oral squamous cell carcinoma, Palliative therapy, Partially ionised plasma, Thermal ionised plasma

INTRODUCTION

Cancer is anticipated as the most prevailing cause of death and the single most significant barrier in increasing life span across the globe in the current period of time, with non communicable diseases contributing to the majority of deaths worldwide. Head and neck carcinoma, oral carcinoma, and other types of carcinoma cause a considerable global threat [1]. According to GLOBOCAN 2018, the global incidence of OSCC is 354,864 cases, with 1,77,384 deaths. With a 5-year survival rate of roughly 50%, OSCC ranks as the sixth most common cancer in the world [2]. The stage of the tumour at presentation, as well as the occurrence of lymph node metastases and distant metastases, impacts the prognosis of patients with OSCC. One-third of patients have stage I, and others have stage IV cancer involving lymph nodes and metastasis [3]. Tumours in the early stages are treated with surgery or radiotherapy and have a good prognosis. Three years after standard treatment, 35 to 55% of patients with OSCC stage IV are disease-free. However, in 30-40% of patients, Locoregional Recurrence (LRR) occurs, and distant metastases occur in 20-30% of instances of OSCC [4]. Many patients are in advanced stages at the time of diagnosis, resulting in high mortality and morbidity, as well as clinical problems that makes clinical management challenging.

Despite no decline in the number of cancer-related deaths, the number of individuals with OSCC has reduced due to improvements in early diagnosis methods and treatment of the disease. Early detection of carcinoma has significantly decreased the number of deaths from the disease, but the success of treatment depends on addressing complications. Advances in cancer research have provided abundant knowledge about cellular processes and molecular biology in OSCC [5]. Early diagnosis, an increase in translation approach, and research in targeted therapy help in improvising the treatment approach for cancer patients. The recommended course of treatment for advanced squamous cell carcinoma is surgery along with adjuvant radiation therapy and/or chemotherapy [6]. The five-year survival rates are low (40-50 percent) and the treatment leads to morbidity [7]. Although chemotherapy has been used to treat metastatic disease but usually, surgery, radiation therapy, and/or chemotherapy are routinely combined to treat LRR [8]. The control of LRR has been quite limited, despite various therapeutic strategies. Addressing the fundamental causes of LRR will therefore improve diagnosis and treatment [9].

Despite recent advancements in cancer treatment, oral squamous cell cancer, in particular, is a fatal and debilitating disease. The life expectancy of patients treated for cancer is still on the lower side which proves to be a driving force for novel research lines in oncology. Cytostatic drug therapy, such as cisplatin, methotrexate, bleomycin, fluorouracil, cetuximab, or docetaxel, is the conventional treatment. This method of chemotherapy has adverse effects in many cases, which are well-known either from basic clinical experience: Anaemia brought on by myelosuppression, infections, haemorrhage, renal failure, neurotoxicity, and lung fibrosis [10]. CAP plasma is a new therapy option that seems to be used mostly as part of a salubrious cancer treatment program to provide comfort to these patients. It is a partially ionised gas that can be applied as a new therapeutic approach in the study of cancer. When provided at a certain concentration, reactive oxygen and nitrogen species, which are produced by physical plasma, destroy cancerous cells [11].

1. COLD ATMOSPHERIC PLASMA (CAP) THERAPY: A NOVEL APPROACH

Plasma is the fourth category of physical state, distinctive from the solid, liquid, and gaseous forms [12]. Since blood plasma is a protein-rich liquid, the term “plasma” in this article refers to the gaseous form of plasma. It is made up of elements such as electrons, cations, anions, gas radicals, and ultraviolet radiation [13]. Plasma treatment is used in medical research to evaluate particular, largely non lethal, and potentially stimulating plasma effects on living cells and tissue [14]. In 2007, the first study of using plasma in cancer treatment was published, proving the silencing of melanoma cells in-vitro following plasma therapy [15]. After that, other research revealed that plasma had anticancer properties in a variety of cancers, including brain, skin, breast, colorectal, lung, cervical, leukaemia, pancreas, liver, and head and neck malignant tumours [16]. CAP, in addition to inhibiting cancer cell development, can also restore chemo-resistant cancer

cells' sensitivite to certain medications [17]. Another illustration is the fact that Tumour Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL)-resistant colorectal cancer cells would become susceptible to the TRAIL treatment when exposed to CAP, suggesting that cold plasma therapy serves a significant role as a palliative treatment for a variety of conditions and cancer types. For instance, Temozolomide (TMZ)-resistant glioblastoma cells have been reverted to TMZ therapy by applying CAP [18]. Another illustration is the fact that TRAIL resistant colorectal cancer cells would become susceptible to the TRAIL treatment when exposed to CAP, suggesting that cold plasma therapy serves a significant role as a palliative treatment for a variety of conditions and cancer types [19].

2. COLD PLASMA DEVICES USED IN MEDICAL APPLICATIONS

A decade, from 1995 to 2004, witnessed the first revolutionary investigations using CAP in the biomedical industry. Dielectric Barrier Discharge (DBD) was first used in research to create pulsed plasma in saline solutions for surgical purposes as well as to inactivate microorganisms on surfaces and in liquids [20]. Since then, numerous laboratories and research facilities across the world have been pursuing applications in wound healing, dentistry, cancer treatment, etc., [20]. Various types of CAP have been discovered like Plasma jets, DBD, nanosecond plasma guns, floating electrode DBD, atmospheric pressure glow discharge torch, plasma brush, micro hollow cathode discharge jet, and microwave plasma torch are types of devices which are used for cold plasma therapy [21].

3. METHODOLOGY OF APPLICATION OF CAP

There are two ways that CAP can be used. The first method is known as "direct" exposure. Since this plasma interacts directly with the biological target in this technique of application, all plasma-produced chemicals try to show an effect on the cells/tissues. Another method is known as "indirect" exposure. Only the plasma's afterglow has been used [20] in the case, or the plasma acts as an activator for a liquid medium first, then cells and tissues are coated with the Plasma-Activated Liquid (PAL). One benefit of this approach is that the PAL can be preserved and utilised again at a later point in time, enabling it a degree of flexibility that direct exposure seems not to [22].

3.1 Direct Cold Atmospheric Plasma (CAP)

Under direct exposure to CAP, nucleic acid and protein components of the cancerous cells are exposed to all plasma agents, including charged particles, photons, electric field, and reactive species [23]. These agents work independently and/or in tandem to create specific biological results. Experiments with several cell lines have shown that, at a specific exposure dose, CAP can selectively destroy cancer cells [24].

3.2 Indirect Cold Atmospheric Plasma (CAP)

Only durable chemical substances that diffuse and solvate into the water have contributed to this type of exposure. Heat, photons, electric fields, ephemeral microorganisms, and electric fields are all destroyed. Plasma-Activated Media (PAM) is created using various culture media and water to create Plasma-Activated Water (PAW) [25]. The process of creating PAM entails exposing a liquid medium to a CAP source, most frequently a plasma plume that lasts for a while from a plasma jet. Eagle's Minimum Essential Medium (EMEM), Dulbecco's Modified Eagle Medium (DMEM), Ringer's Lactate solution (RL), and Roswell Park Memorial Institute medium (RPMI) were utilised, along with serum (e.g., bovine serum), glutamine, and antibiotics (e.g., Penicillin/Streptomycin combination) [26]. A 24-well plate can be used to make PAM by mixing a few mL of fresh cell culture medium into each well. CAP can be used to treat each well for a set amount of time, resulting in various PAM with different "strengths". The more media is getting treated with CAP, the more effective it is at killing cancerous cells [27].

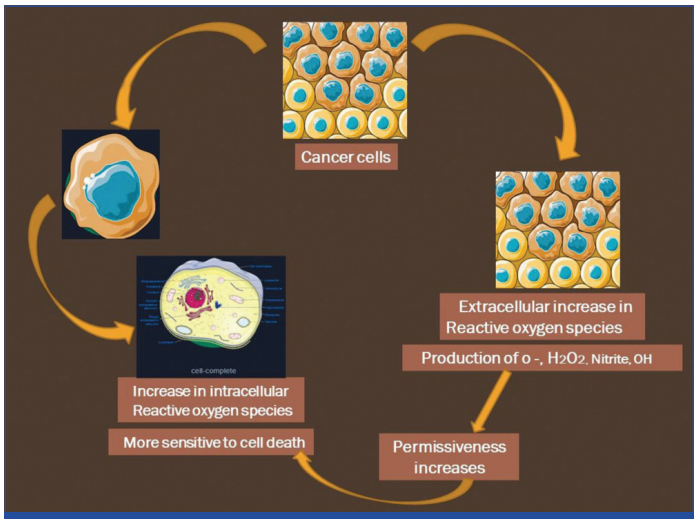
4. THE BASIC PRINCIPLE FOR CAP THERAPY APPLICATIONS

To prevent thermal degradation when applied directly or inside the human (or animal) body for therapeutic purposes, plasmas should be stable reproducible in open atmosphere conditions and cold (40°C) at the tissue contact zone [28].

4.1 Hypothesis on the Principle of Action of CAP on Cancer Cells

First hypothesis

Cancer cells because of altered metabolism and mitochondrial malfunction [Table/Fig-1] frequently create more intracellular Reactive Oxygen Species (ROS) than non malignant cells [29]. Increased intracellular ROS in cancer cells have been shown to make them more sensitive to cell death caused by extracellular (Ex-ROS) in some investigations. Extracellular ROS formed by plasma are superoxide anion, hydrogen peroxide, peroxynitrite, nitrite, nitrate, hydroxyl radicals, atomic oxygen, ozone, and singlet delta oxygen [30].



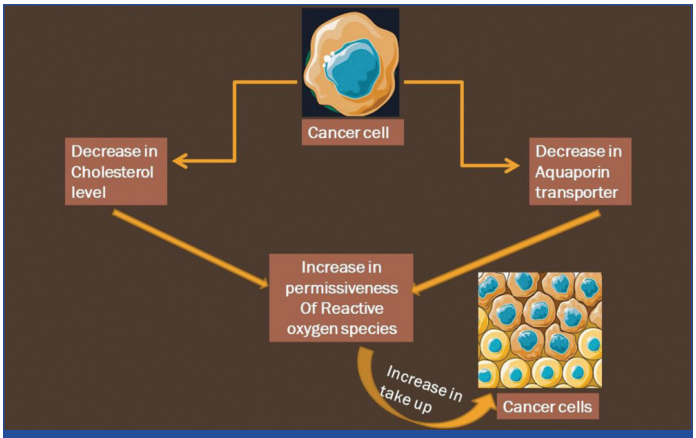
[Table/Fig-1]: Altered metabolism and mitochondrial malfunction in cancer cells.

Second hypothesis

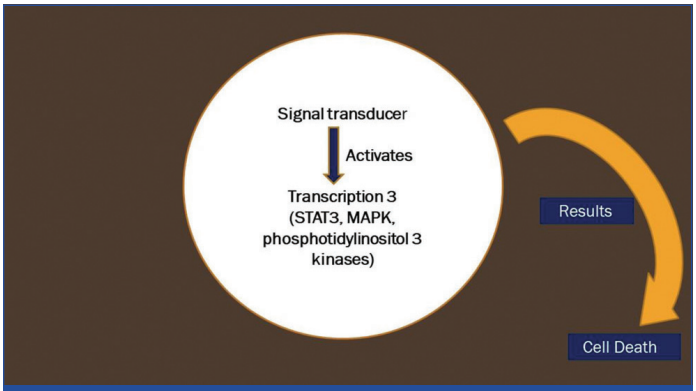
While comparing cancer cells to non malignant cells, aquaporin transporters and lower cholesterol levels in the membrane increase the permissiveness of ROS through the cell membrane of the cancerous cells, most specifically by peroxidation of lipids. As a result, more plasma-derived ROS are taken by cancer cells, leading to increased cell death [Table/Fig-2a] [31]. The signaling pathway which takes place inside the cell such as Signal Transducer and Activator of Transcription 3 (STAT3), MAP Kinase (MAPK), and phosphatidylinositol 3 kinases through AKT, resulting in apoptosis (protein kinase B). Because of their special attributes, plasma treatment can selectively target OSCC or any other cancer [Table/Fig-2b] [32].

5. MECHANISM OF ACTION OF COLD PLASMA THERAPY ON ORAL CANCER

Interfering with the cell cycle is one strategy to target cancer cells. Unlike normal cells, cancer cells multiply more quickly. Combination therapy is the most efficacious because they target cancer cell biology through several signaling pathways, creating a harmonious effect [33]. The major aim of cold plasma therapy is to make these cancerous cells pass through a process

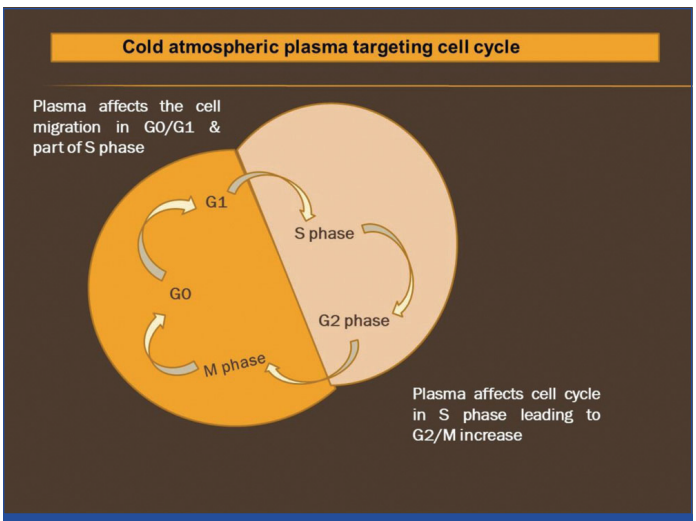


[Table/Fig-2a]: Extracellular-mechanism of action (Extracellular).



[Table/Fig-2b]: Cell signaling pathway inside the cell.

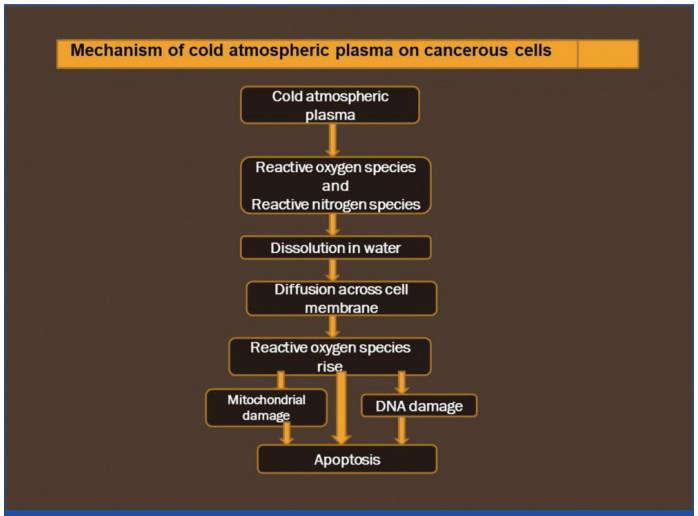
known as "the cell death process", or apoptosis. CAPs, which have newly been developed, could be used as an alternative therapy for cancer. If CAP is applied locally, it helps in reducing the size of the tumour [34]. According to one of the in-vitro studies, the use of CAP causes apoptosis- the death of cancer cells which stops the excessive cell division and proliferation that are the primary causes of tumours and cancer [35]. Previous studies have reported that the ROS play a major role in cell response to CAP treatment in-vitro and in-vivo [36-39]. In another study, the hypothesis for the mechanism of action of cold plasma therapy on oral cancer cells was acting on the S phase of the cell cycle [Table/Fig-3]. Because a higher percentage of cancer cells are in the S phase, they are more vulnerable to the impacts of CAP [40]. Disturbances in cell matrix, cell-cell adhesion, or terminal differentiation, which lead to homeostatic imbalance and are responsible for carcinogenesis in oral cancer, skin cancer, and other cancers, are the major culprit in cancer formation [41].



[Table/Fig-3]: Cold Atmospheric Plasma (CAP) targeting cell cycle.

5.1 Anticancer role of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) generated by CAP [Table/Fig-4]

The ROS and RNS produced by CAP were first dissolved in the medium or Phosphate Buffer Saline (PBS). When the CAP treatment was done, most cancer cells were exposed to media. In contrast, in a few research and treatments, CAP treated media was applied directly to cancer cells. The direct CAP therapy and the indirect CAP treatment are thought to be nearly comparable while considering the involvement of ROS dissolved in the media. Pyruvate is adequate to mitigate the toxicity of the CAP-treated medium on cancer cells by mixing the dissolved ROS in the media. The ROS and RNS produced by cold atmospheric plasma (CAP) will permeate through the cellular membrane. Peroxynitrite ($ONOO^-$), singlet oxygen ($_1O^2$), hydroxyl radical (.OH), superoxide anion ($O2^-$), and hydrogen peroxide are all scavengers of ROS in the nordihydroguaiarectic acid (NDGA) (H_2O_2) [42]. As a result, nordihydroguaiarectic acid (NDGA) may not have a direct effect on the transport of reactive species through the transmembrane.



[Table/Fig-4]: Mechanism of Cold Atmospheric Plasma (CAP) on cancerous cell.

Despite this, cancer cells that have been treated with CAP have been proven to increase ROS [43]. As a result, subsequent CAP treatment, and transmembrane diffusion of reactive species should be envisaged. Even though most ROS and RNS originating in CAP are polar or charged molecules specialised membrane proteins must facilitate ROS and RNS transmembrane diffusion [44]. When exogenous ROS enter, they consume and weaken the intracellular antioxidant system which is primarily made up of tiny molecules like Glutathione (GSH) and reduced nicotinamide adenine dinucleotide phosphate NADP/(NADPH). In the cancer cells which are treated by CAP, the ratios of GSH and Nicotinamide adenine dinucleotide phosphate are reduced [45]. There is also a decrease in the expression of superoxide dismutase and catalase. As a result of the impaired antioxidant system, the increase in ROS is accelerated. Damage to mitochondria and Deoxyribonucleic Acid (DNA) is always a direct result of an increase in ROS. Apoptosis is triggered by both mitochondrial and DNA damage.

The major cellular response to CAP therapy has been identified as an increase in ROS [46]. The number of basal ROS in cancer cells is assumed to be greater than in normal cells as the cancer cells have a higher metabolism. When cancer cells are exposed to exogenous ROS stress, the level of ROS in the cells crosses a threshold more abruptly than in normal cells, triggering apoptosis, an anticancer strategy [47]. The disparity in ROS behavior between cancer cells and normal cells might explain how the cancer cells have higher apoptotic and mortality rates than normal cells. The substantial

ROS rise, on the other hand, occurs only in cancer cells, not in normal cells. Apoptosis is induced by cold plasma therapy, which inhibits cells from proliferation.

5.2 The Epigenetic Approach of Cold Plasma Therapy on Cancerous Cells

There is still a dearth of understanding that how CAP affects epigenetic alterations and impacts non coding RNA activity and expression [48]. Furthermore, there is still a debate on whether CAP has a direct effect on DNA damage [49]. Tumour cells are more likely to enter the S phase of the cell cycle. DNA gets unwinded in the S phase, making it more vulnerable to ROS and RNS. While reactive species and radicals created by CAP might cause oxidative stress in cancer cells [50]. The response of these cancer cells varies depending on the concentration of the stressor. Even though epigenetic alterations do not affect DNA sequence directly, they do have an impact on gene expression [51]. The fundamental goal of epigenetic modifications is to ensure the maintenance of specific cell types and to specialise individual cells' biological tasks [52]. These epigenetic alterations are classified into two categories: those that impact nucleic acid methylation levels and those that affect histone proteins [53]. Finally, cancer cells' epigenetic state is frequently harmed. Many researchers have studied the effect of CAP on various cancer cell lines.

The effect of CAP on the MCF-7 and MDA-MB-231 breast cancer (BC) cell lines was discovered by Bin PS et al., [54]. These researchers examined the variations in the methylation levels of specific CpGs in Alu elements, which are generally hypermethylated in healthy tissues but frequently hypomethylated in malignancy [55]. Following exposure to CAP, their initial pyrosequencing results revealed minor hypo-methylation in the targeted Alu region, but only in the MDA-MB-231 cells. Although the decrease in methylation rate from 23.4 to 20.3% was statistically significant at p-value <0.05, it is debatable if this is noteworthy because no statistically significant alterations were observed in the second cell line, and a difference of three percent is not significant. However, in the same cell lines, whole-genome methylation microarray analysis yielded more significant results. Hou J et al., examined how the length of CAP exposure impacted the overall CAP effect on A549 small lung cancer cells [56]. The authors applied CAP to the cells for one and three minutes, then assessed changes in gene expression 2, 4, and 6 hours later. The researchers discovered that cells exposed to CAP for one minute had a different effect on the process of distinct gene groups than those exposed to three minutes. SCC-15 squamous cell carcinoma cell lines and HGF-1 Human gingival fibroblast cell lines were treated with CAP and cisplatin treatments and then both of them were given together to examine the synergistic effect of CAP and cisplatin together [57]. The vitality of tumourous and control cell lines was reduced by cisplatin alone which is dependent on the dose. Nonetheless, when CAP was added, the decline in tumour-cell viability was higher.

Furthermore, when compared to the SCC-15 tumour cell lines, the decrease in fibroblast cell viability was much smaller in all solitary cisplatin and CAP treatments, as well as in their combination application. The application of 3 μM cisplatin to tumour cells resulted in the loss of vitality in 50% of tumour cells, but only 10% of fibroblast viability. Only around 50% of tumourous cells were viable after three minutes of CAP therapy, however, over 70% of fibroblasts stayed viable. For the intended synergistic impact on SCC-15 cells, a combination of 1 μM cisplatin with 3-min CAP treatment or 3 μM cisplatin with 1-min CAP therapy was shown to be best [58]. When CAP and cisplatin were given together, they had a greater influence on the expression of several apoptosis-related genes than when cisplatin or CAP was given separately. PTEN, as well as the genes that code for Caspase 9 and p53, are among these genes. In cancer cells, the effect was more significant than in fibroblasts [59].

As a result of the preceding investigations, it appears that CAP exposure can result in the following outcomes: (a) High amounts of the ROS produced by CAP directly induce DNA damage in cancer cells, which leads to apoptosis or other types of cell death; or (b) CAP causes apoptosis, which leads to DNA fragmentation and increasing levels of H2AX phosphorylation. The CAP-produced ROS, on the other hand, play a crucial role in both cases. Because of the altered membrane structure of cancer cells, and notably their membrane lipid structure, ROS can permeate them more easily [60]. Furthermore, tumour cells have greater levels of $\bullet O^{2-}$ super-oxide in their extracellular matrix. Even though multiple other research has correlated CAP exposure to the occurrence of DNA damage [60]. Studies on use of CAP for the treatment of oral cancer have been tabulated I=in [Table/Fig-5] [30,57,61,62].

Author's name and year	Place of study	Objective of the study	Inference of the study
Guerrero-Preston R et al., 2014 [61]	USA	To explore the potential use of CAP as a minimally invasive surgical approach in Head and Neck Squamous Cell Carcinoma (HNSCC)	Results show cold plasma application selectively impairs HNSCC cell lines through non apoptotic mechanisms, while having a minimal effect on normal oral cavity epithelial cell lines.
Bauer G et al., 2019 [30]	Freiburg, Germany	To understand the selective in-vitro antitumour mechanisms of Cold Atmospheric Plasma (CAP) and Plasma-Activated Media (PAM) which follows a sequential multistep process.	The conclusion from these experiments is that tumour cell-generated RONS play a major role in inactivating protective catalase, depleting glutathione, and establishing apoptosis-inducing RONS signaling. CAP or PAM exposure only triggers this response by initially inactivating a small percentage of protective membrane-associated catalase molecules on tumour cells.
Lee CM, et al., 2020 [57]	Gwangju, Korea	To examine the synergistic effect of the combination of CAP and cisplatin-mediated chemotherapy of Oral Squamous Cell Carcinoma (OSCC) in-vitro.	The synergistic anticancer effect of cisplatin and CAP treatment was examined using SCC-15 and HGF-1 cells in-vitro. The viability of SCC-15 cells was inhibited by cisplatin in a dose-dependent manner and/or CAP treatment time.
Lee JH et al., 2016 [62]	Korea	To investigate the effects of CAP plasma-induced radicals on the Epidermal Growth Factor Receptor (EGFR), which is overexpressed by Oral Squamous Cell Carcinoma (OSCC), to determine the underlying mechanism of selective killing.	Nitric oxide scavenger pretreatment in cell culture media before CAP treatment rescued the above degradation and dysfunction of the EGFR as well as the killing effect in Oral Squamous Cell Carcinoma (OSCC). CAP may be a promising cancer treatment method by inducing EGFR dysfunction in EGFR-overexpressing Oral Squamous Cell Carcinoma (OSCC) via nitric oxide radicals.

[Table/Fig-5]: Studies including Cold Atmospheric Plasma (CAP) used for the treatment of oral cancer.

6. CAP AND SELECTIVE ANTICANCER MECHANISM

The fundamental advantage of CAP over most anticancer techniques, such as chemotherapy and radiotherapy, is its selective anticancer capability, which has been proven in several cancer cell lines [32]. CAP seems to impede the growth of cancer cells rather than similar normal cells under the same experimental settings [61] by triggering more apoptosis in cancer cells than in normal cells [62]. One of the main challenges in this subject is figuring out how to create a selective anticancer mechanism. This selective action could be due to the well-known fact that when cancer cells are exposed to CAP, a considerable increase in ROS occurs in cancer cells but not in normal cells [63].

7. SAFETY CONCERNS ASSOCIATED WITH CAP IN BIOMEDICAL APPLICATION

A German industrial norm proposal known as DIN-specification 91315 was created as part of an endeavor to examine any potential plasma supply for a biological application involving patient contact. This method, which is often employed for such studies, outlines the potential physical, chemical, and biological risk aspects as well as performance standards from plasma devices in the application [64]. A study done by Arndt S et al., investigated wound healing using CAP pressure. In this study, they unearthed the risk of CAP about the potential damage of DNA and mutagenesis on exposure to CAP in-vitro. This is one of the side-effects or risks associated with the use of CAP pressure [65]. In another study done by Scully C et al., it showed that cell type or different plasma sources if used for a short duration had a stimulating effect i.e., the proliferation of cells and migration, which might accelerate the progression of cancer cells [66]. The production of CAP completely depends on the voltage, electric, and magnetic field associated with the plasma jet. The voltage and electricity for the production of CAP are according to the safety and efficiency of CAP on cancer cells [67].

8. SIDE-EFFECTS OF CAP IN TREATING ORAL CANCER

CAP's detrimental plasma effects on cells result either in cellular repair processes or in the induction of programmed cell death (apoptosis) [68]. Several studies using well-established and accepted experimental procedures have proven that CAP treatment does not cause an increased risk for genotoxicity [69-71]. CAP does not have any lethal or life-threatening side-effects. The side-effects the patient experience are very mild like bad taste, bleeding, and nausea which will subside on their own. At present and from a clinical point of view, there is no risk of severe side-effects obvious when applying CAP in cancer patients for palliation [72].

The line of treatment for OSCC is radiotherapy and chemotherapy. These treatments are always associated with lethal side-effects and may be responsible for genotoxicity but the discovery of CAP can be a great boon to medical science and specifically in oncology as it has fewer side-effects associated with this palliative treatment [73]. The pathway followed by CAP is ROS which is responsible for oxidative stress. The mechanism of action of CAP is in such a way that it causes apoptosis only in the cancerous cell, unlike chemotherapy and radiotherapy [22]. Many studies have already proved that it is useful in wound healing, dental treatment, actinic keratosis, etc., [74,75]. In this review, we have discussed CAP therapy in oral cancer and its various pathways which play a major role in the apoptosis of cancerous cells.

In the future aspects, cold plasma therapy can be used as a successful palliative therapy used with radiotherapy and chemotherapy. Many researchers have noticed the synergistic effect of cold atmospheric plasm therapy with chemotherapeutic drugs. Head and neck cancers have been associated with poor cosmetic outcomes. Traditional surgery, as well as chemotherapy and radiotherapy, may have psychological consequences due to unpleasant changes in appearance, as well as physiologic consequences due to functioning problems of the affected facial organs [76]. Because of its mild but effective selectivity on cancer cells, such patients may benefit from CAP treatment.

CONCLUSION(S)

The fourth phase i.e., CAP therapy has been proven as a useful discovery in oncology. The discovery of CAP's anticancer properties, as well as its preclinical and clinical efficacy, has the potential to pave the way for the establishment of a wide range of synergistic and personalised plasma-enabled treatments. These medicines have the potential to not only provide a mild but effective cancer monotherapy with a broad therapeutic window and good selectivity but also to enhance existing therapies toward safer, more effective treatment modalities. The ability of CAP to sensitise resistant cancer cells to conventional therapies needs special attention, and more research should be done to understand potential synergistic and antagonistic events that may occur when CAP is used as a palliative treatment.

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