A Review on Melanoma Treatment by Cold Atmospheric Plasma

By

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled “Melanoma treatment by cold atmospheric plasma” submitted by Alif Hasan Pranto (18346009) of Summer 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

The thesis was done without unethical work. No human or animal tests are involved in this study.

Abstract

Melanoma is the deadliest form of skin cancer. According to the American Cancer Society, melanoma kills 158,000 people annually. No melanoma vaccine has been approved. "Wide local excision" surgery is used to treat early-stage melanoma. The surgeon removes the tumor and a margin of normal skin. The surgeon will perform a sentinel lymph node biopsy to see if the melanoma has spread. Cold atmospheric plasma (CAP) generates two components: physical and chemical factors. Thermal, UV, and electromagnetic radiation destroy melanoma. RONS—reactive oxygen and nitrogen species—is another factor. Both factors increase immune cell-like T cells and helper T cells like CTD-4 and CTD-8 while decreasing B16F10. To treat B16F10 cell growth, cold atmospheric plasma changes the composition of tyrosine (tyr) and tryptophan (trp). Also, make changes in extracellular tissue pH to induce anti-cancer effects. Cold atmospheric plasma is affordable and easy to utilize, making it ideal for treating cancer cells.

Keywords: Melanoma; cold atmospheric plasma; reactive oxygen and nitrogen species; T cells

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Dedication

I dedicate this to my parents, for your constant love and support.

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List of Acronyms

CAP	Cold atmospheric plasma
RONS	Reactive oxygen and nitrogen species
PAPBS	Phosphate-buffered saline

Glossary

Cold atmospheric plasma:	An ionized gas.
Melanoma:	The most serious type of skin cancer.
Phosphate-buffered saline:	A buffer solution (pH ~ 7.4).
T cells:	A type of white blood cell.

Chapter 1

Introduction

1.1 Plasma

Plasma is the condition of substance that has a high energy level and is characterized by the existence of ions and free electrons. It is the fourth stage of the matter, and it is the condition ion that the matter remains. Plasmas are considered to be the fourth state of matter because they are different from the other three states of matter: solid, liquid, and gas. While solids have a defined shape and volume and fill their container while liquids take on the shape of the container they are contained inside., plasmas do not have a defined shape or volume and are often described as a "fluid" of ions and electrons. the sun and lightning are examples of plasmas. The sun is a plasma because it is composed of highly charged ions and free electrons, which makes it conductive and able to generate magnetic fields. Lightning is also plasma because it is created when an electric discharge ionizes the air and produces a high-energy state of matter composed of ions and free electrons. When an electron de-ionizes from a molecule's outer shell. This molecule is capable of producing energy and enhancing electric conductivity (Chen, 2022).

The plasma can be split into two categories based on the electron or proton temperature. Those in thermodynamic equilibrium are local thermodynamic equilibrium (LTE), plasma with a temperature that is really high, non-local thermodynamic equilibrium, plasma that is relatively near to the temperature of a typical living room.

1.2 Cold atmospheric plasma (Non-thermal)

Cold Atmospheric Plasma (CAP) refers to one of the species of plasma that can be produced in the air at atmospheric pressure and temperatures that are low compared to traditional plasmas. Plasma, a fourth state of matter, is created by ionizing a gas, which results in the presence of positively and negatively charged particles. CAP is generated using high voltage and low- frequency electrical discharge, which ionizes the surrounding air, creating plasma (Chen, 2022).

The low temperature of CAP makes it an attractive option for various industrial and medical applications. CAP has been found to have a variety of effects on materials and biological systems, including surface sterilization, degradation of pollutants, and treatment of cancer (Daeschlein et al., 2013).

The capability of CAP to sterilize surfaces is one of the most important applications of this technology. However, it has been discovered that CAP is able to successfully destroy a wide variety of microorganisms, including bacteria, whereas traditional sterilization procedures, such asheat and chemical treatments, can cause harm to delicate surfaces. viruses, and fungi, without damaging the surfaces. This makes it an attractive option for sterilizing medical instruments and other sensitive equipment (Daeschlein et al., 2013).

Additionally, cancer patients have been treated with CAP in some cases. The high-energy particles produced by CAP have been shown to effectively kill cancer cells, making it a promising alternative to traditional treatments such as radiation and chemotherapy. Additionally, because CAP does not produce harmful side effects like traditional treatments, it is considered a safer option for cancer patients. Furthermore, CAP has been utilized in the treatment of a variety of additional conditions, such as wound healing, dermatitis, and acne. It has been demonstrated that the high-

energy particles that are generated by CAP promote healing by encouraging the formation of new cells and tissues. This makes CAP an attractive option for the treatment of various skin conditions, as well as for wound healing (Daeschlein et al., 2013).

1.3 Melanoma

Melanoma is a form of skin cancer that develops in the cells that are in charge of pigment production. Melanomas tend to spread aggressively (melanocytes). It can appear as a new mole or as a change in an existing mole, and it is often characterized by irregular borders, uneven coloration, and asymmetry. Melanoma can become a life-threatening condition if it is allowed to progress untreated and spread to other tissues throughout the body. The worldwide death rate from melanoma has been increasing over the years and is a major public health concern.

According to the World Health Organization (WHO), Melanoma ranks fifth among the most common cancers seen in males and sixth among the most common cancers found in females. Among men, it is the fifth most common disease. In addition to this, the rate at which people develop melanoma has been gradually growing, especially in countries with high levels of ultraviolet (UV) radiation exposure. The highest incidence rates of melanoma are found in Australia, New Zealand, and North America, where the incidence rate is more than 20 cases per 100,000 population. The death rate from melanoma is directly related to the amount of severity of the disorder at the time of the diagnosis. If detected early, melanoma is often treatable with a high success rate. In the event that the infection has spread to other portions of the body, The prognosis is not good, with only a 25% chance of survival after five years. of only 15-20%. This is why early detection is critical to improving outcomes and reducing the death rate from melanoma (Daeschlein et al., 2013).

One of the main factors contributing to the high death rate from melanoma is the lack of public awareness about the importance of early detection and skin protection. Many people are unaware of the dangers of excessive sun exposure and the importance of protecting their skin from the harmful effects of UV radiation. In addition, there is often a delay in seeking medical attention for skin changes, which can lead to a later stage of the disease and a poorer prognosis.

Another factor contributing to the high death rate from melanoma is the limited access to effective treatment options in many countries. In many parts of the world, there is a lack of adequate medical facilities, trained healthcare professionals, and financial resources to provide the necessary treatment for melanoma. This can lead to a higher death rate, particularly in low- and middle-income countries (Gan et al., 2021).

1.4 Cold atmospheric plasma in vivo response

Representative in vivo demonstrations on subcutaneous xenografted tumor models

Table 1: In vivo responses of cancer cells on subcutaneous xenografted tumor models (Daeschlein et al., 2013).

Years	Tumor Types	Tumor Size	Survival Rate	Tumor Diagnostics
2010	Glioblastoma	↓	N/A	photosynthetic bacteria
2010	Bladder cancer	↓	↑	measuring tissue size
2011	Glioblastoma	↓	↑	photosynthetic bacteria
2012	Pancreatic carcinoma	↓	N/A	photosynthetic bacteria

2012	Glioblastoma	↓	N/A	photosynthetic bacteria
2013	Neuroblastoma	↓	↑	measuring tissue size
2014	Melanoma	↓	N/A	measuring tissue size
2014	Head and neck cancer	↓	N/A	measuring tissue size
2015	Melanoma	↓	↑	measuring tissue size
2015	Endometrioid adenocarcinoma	↓	N/A	measuring tissue size
2016	Glioblastoma	↓	N/A	measuring tissue size
2016	Breast cancer	↓	N/A	
2017	Melanoma	↓	N/A	photosynthetic bacteria
2018	Colorectal tumor	↓	N/A	measuring tissue size

1.5 Cold atmospheric plasma in vitro response

Table 2: In vitro responses of cancer cells using CAP (Chen, 2022).

Cancer cells in vitro responses to treatment utilizing cold atmospheric plasma	**Years**
Apoptosis	2004
Growth Suppression	2007
Damage to the cytoskeleton	2009
Selected Cell Death	2010

Interrupted cell cycle	2010
DNA and nuclear damage	2010
Damage to the mitochondria	2010
Rise of Intracellular ROS	2011
Chemically-based Drug Sensitization	2013
Increase in Certain Intracellular ROS	2013
Senescence	2013
Immune-related cell death	2015
Hydrogen peroxide generation in cells	2017
Cell Death Involved in Autophagy	2017
Phenomena of Activation	2018
Necrosis that is physically triggered	2020
Proptosis	2020
Physically-based Drug Sensitization	2021

1.6 Cold atmospheric plasma implementation to treat cancer

Traditional treatments for melanoma include surgery, chemotherapy, and targeted gene therapy. However, these treatments can have significant side effects and are not always effective, especially in the advanced stages of the disease.

Cold atmospheric plasma (CAP) is a relatively new technology that has shown promising results in treating melanoma. CAP is a type of plasma that is generated at atmospheric pressure and low temperatures. Unlike other types of plasma, it does not generate harmful heat and radiation, making

it a safer alternative for medical applications. The capacity of CAP to selectively target malignant cells while minimizing damage to healthy tissue is one of the most significant advantages of CAP as a treatment for melanoma. This is due to the fact that the plasma produces reactive species that are capable of penetrating the cell membrane, which ultimately results in damage and death to the cells. Although the precise method by which this takes place is not completely understood, it has been demonstrated that CAP is capable of inducing apoptosis, also known as programmed cell death, in malignant cells while having no effect on healthy cells that much (Gan et al., 2021).

In addition, CAP has been shown to enhance the efficacy of traditional treatments such as chemotherapy and targeted gene therapy. This is because the plasma can increase the amount when the cell membrane is permeable, allowing drugs to penetrate deeper into the cancerous cells and improve the therapeutic efficacy. Furthermore, CAP can also activate the immune system, helping the body to naturally fight against cancerous cells.

In both in vitro and in vivo models of the disease, research has demonstrated that CAP is an effective treatment for melanoma. For instance, a study that was published in the "Journal of Investigative Dermatology'' discovered that CAP was able to dramatically slow down the expansion of melanoma tumors in mice, without causing any notable adverse effects.

In general, the use of cold atmospheric plasma as a potential alternative therapy for the treatment of melanoma is very encouraging. Patients may find it to be a desirable option because of its capacity to selectively target malignant cells while simultaneously boosting the effectiveness of conventional treatments, as well as since it does not cause any adverse side effects. However, additional research is required to completely understand the mechanism of action of CAP and to

determine its safety and efficacy in bigger clinical studies. This can only be accomplished by further investigation (Chen, 2022).

It is essential to keep in mind that the CAP is still in the preliminary stages of research and development and that it is not yet readily accessible for clinical applications. Patients diagnosed with melanoma should discuss their treatment options with their primary care physician in order to select the modality that is most suited to their specific requirements (Gandhirajan et al., 2018).

In recent years, there has been a rise in interest in a novel and cutting-edge treatment option for melanoma known as cold atmospheric plasma (CAP). This treatment modality is considered to be relatively new. Melanoma is a particularly difficult form of skin cancer to treat because it is an extremely aggressive form of the disease. Conventional treatments for melanoma, such as chemotherapy, targeted gene therapy, and surgery, each have their own set of limitations as well as the potential for adverse effects. CAP is a possible answer to this problem, which is where it comes in (Gandhirajan et al., 2018).

The CAP treatment is a non-invasive method that makes use of plasma, which is the fourth state of matter and is produced by applying high voltage to a gas mixture. The plasma that is produced in this manner is highly reactive and contains a significant number of active species that are able to engage in interactions with cells and tissues. In the case of melanoma, CAP can either be used to directly target and eliminate cancer cells, or it can be utilized to boost the effectiveness of other treatments, such as chemotherapy and targeted gene therapy.

One of the main advantages of CAP is that it is non-invasive. Unlike surgery, which requires incisions and can leave scars, CAP does not require incisions or stitches and leaves no visible signs of treatment. This is particularly important for patients with melanoma located in sensitive areas

like the face or hands. Additionally, CAP is pain-free, reducing the anxiety and discomfort associated with more invasive treatments.

Furthermore, CAP can be administered multiple times, which is a potential benefit for patients with melanoma has returned after treatment. Another advantage of CAP is that it is relatively fast and convenient. Treatment sessions typically last only a few minutes and do not require an extended recovery period. This is in contrast to surgery, which can require several weeks of recovery time and limit a patient's ability to perform normal activities. Traditional treatments for melanoma, such as chemotherapy and targeted gene therapy, may not be as effective as CAP, which may make it a more desirable option for those seeking treatment. For instance, CAP has been demonstrated to improve the efficacy of chemotherapy medications in a number of studies. It does this by increasing the amount of drugs that are

taken up by cancer cells while simultaneously reducing the toxicity of the drugs to normal cells. In a similar vein, it has been demonstrated that CAP can boost the expression of therapeutic genesin cancer cells, so improving the effectiveness of targeted gene therapy.

While CAP is a promising treatment option for melanoma, It is essential to keep in mind that it is still a relatively new and rapidly evolving field. There is limited clinical evidence to support the use of CAP for melanoma, and more research is needed to fully understand its benefits and limitations. Additionally, CAP is not yet widely available and may not be covered by insurance, which can limit its accessibility for some patients (Daeschlein et al., 2013).

In conclusion, CAP is an innovative and promising treatment option for melanoma that offers several advantages over traditional treatments like chemotherapy, targeted gene therapy, and surgery. While more research is needed to fully understand its benefits and limitations, CAP holds

great potential as a gentler, faster, and more effective alternative for certain patients with melanoma. Whether or not CAP is the right choice for a particular patient will depend on their individual needs and preferences, as well as the specific characteristics of their melanoma (Gandhirajan et al., 2018)

1.7 Objective

Cold Atmospheric Plasma is a new technology and promising approach to treating melanoma. Cold Atmospheric plasma will counter all the traditional treatments known for years over the years like chemotherapy and lymph node removal surgery. Because this traditional has so many side effects like hair loss, mouth soreness, fatigue, loss of appetite. Nerve damage, skin changes and fertility problem. CAP is a safe process to treat melanoma during the therapy process it doesn't damage healthy cells because it is a non- thermal and non-invasive process so the main objective of CAP is to provide maximum efficacy in cancer treatment.

1.8 Methodology

The article was retrieved from PubMed, which was searched in the database with key-words. Articles from the last 20 years were included (directly or indirectly integrated). Moreover, non-English and peered articles were excluded immediately.

Keywords: "Cold atmospheric plasma" "melanoma treatment"

Chapter 2

Mechanism

2.1 Melanoma treatment using cold atmospheric plasma

“Cold atmospheric plasma” (CAP) is a promising novel approach to the treatment of melanoma, a type of skin cancer. CAP is a non-thermal, non-invasive form of plasma that is generated by ionizing a mixture of gases at atmospheric pressure. The plasma is composed of electrons, ions, and reactive oxygen, and nitrogen species are examples of numerous reactive species that have the potential to interact with biological systems, including cancer cells. These interactions can be beneficial or harmful.

It has been demonstrated that CAP can promote cell death, decrease cell proliferation, and alter the immune system, all of which are important anti-cancer mechanisms. In the context of preclinical research, it has been demonstrated that CAP is effective against a variety of cancers, Melanoma is one of the cancers. It is assumed that the formation of reactive oxygen and nitrogen species is one of the ways in which CAP exerts its anti-cancer actions in the body. These species are recognized to have the ability to damage cancer cells, trigger cell death, and affect the immune system, all of which are related to cancer treatment. Additionally, it has been demonstrated that CAP can cause cancer cells to undergo oxidative stress, which can result in an increased rate of cell death (Gandhirajan et al., 2018).

It has also been demonstrated that CAP has an influence on the immune system, which is important for the treatment of melanoma because it plays a part in the disease. For instance, it has been demonstrated that CAP can boost the activation of immune cells such as natural killer cells, which are crucial to the process of eliminating cancer cells and preventing their return. Additionally, it

has been demonstrated that CAP has an effect on the generation of cytokines and chemokines, both of which can affect the immune system and increase its capacity to attack cancer cells.

It is assumed that CAP is able to exercise its anti-cancer effects through the inhibition of angiogenesis, which is the process by which new blood vessels are generated. This is a further mechanism by which CAP is able to do so. Inhibition of angiogenesis is an essential part of the therapy of cancer because it halts the progression of the disease and stops it from spreading to other parts of the body. It has been demonstrated that CAP can decrease angiogenesis both in vitro and in animal models, which may be one of the factors that contributes to its effectiveness against melanoma.

Despite these encouraging preclinical results, CAP has not been widely examined in human studies to determine whether or not it is effective in the treatment of melanoma. The use of CAP in the treatment of melanoma has only recently been the subject of a limited number of clinical investigations, and the results of these trials have been inconclusive. In some studies, patients experienced a reduction in the size of their tumors as well as an improvement in their overall survival rates. On the other hand, in other studies, there was no evidence that the treatment was significantly effective.

In conclusion, a wide variety of anti-cancer impacts that have been demonstrated in preclinical trials, CAP is an exciting new therapy option that is being considered for the treatment of melanoma. Although the efficacy of CAP in the treatment of melanoma has not been widely examined in human trials, the results that have been obtained to this point have been promising, with some patients experiencing a reduction in tumor size and an improvement in their overall

survival. To completely analyze. further clinical tests are necessary to determine the efficacy of CAP in the treatment of melanoma as well as the optimal treatment regimen (Yadav et al., 2020).

2.2 Physical factor of CAP

Therapy via the CAP directly (physical factor) utilization of (CAP) as a treatment for melanoma by making advantage of the plasma's physical properties, such as non-thermal radiation, ultraviolet radiation, and electromagnetic waves, in order to destroy cancer cells and use the plasma as a treatment for melanoma. (CAP) physical factor generates energetic species like as ions, electrons, and protons among other species.

During the early phase, first bubbling commences, which also forms some holes while simultaneously causing the cytosol to begin to decrease. The minute bubbles gradually expose their greater size and escape through the minuscule pores that have been generated as a result of the cytosol aggregation process. When compared to the earlier stage, the cytosol becomes significantly less expansive. After then, a separation from the cell membrane begins to occur between the bubbles. The nucleus components of the cell membrane have been destroyed, which has led to the death of the cell, and detachment is how it leads to the death of the cell being caused by a physical trigger. It has been demonstrated that the physical factor approach can successfully reduce the size of existing melanoma tumors and stop the development of new ones. This is achieved by having both anti proliferative and apoptotic effects on cancer cells, which ultimately results in a considerable reduction in the number of cancer cells in the body. As a consequence of which the cells will eventually perish as a result of physical triggers (Yadav et al., 2020).

2.3 Chemical factor of CAP

Indirect therapy provided by the CAP (chemical factor) utilization of (CAP) as a treatment for melanoma by making use of the chemical qualities of the plasma, such as (RONS) and liquid Phosphate-buffered saline (PAPBS), which are administered via injection. (CAP) Chemical factor Produce oxygen and nitrogen species that are capable of reacting (RONS). generating extracellular tissue more acidic, chemical agents help to maintain the pH level between extracellular tissues and the microenvironment of tumor tissue. This, in turn, promotes the development of anti-cancer activities. (RONS) has the potential to influence the levels of the amino acids tyrosine (Tyr) and tryptophan (Trp). These alterations may take place as a result of the plasma's ionization and oxidation reactions, which are both capable of producing them. Phosphate-buffered saline (PAPBS), a chemical-based treatment, causes microbial damage to cells and assists in the induction of apoptosis and necrosis via physical factors; nevertheless, indirect plasma treatment is unable to cause cell death directly.

2.4 Chemical factor and physical factor comparison of CAP

Table: 3 Comparison of chemical and physical factors of CAP (Gandhirajan et al., 2018)

CAP direct treatment (physical factor)	**CAP indirect treatment (Chemical factor)**
Utilization of (CAP) as a treatment for melanoma by making use of the plasma's physical features, such as non-thermal radiation, UV radiation, and electromagnetic waves, in order to kill cancer cells.	Use of (CAP) to treat melanoma by utilizing the Chemical properties of the plasma such as (RONS) and liquid Phosphate-buffered saline (PAPBS) delivered by injection.
produces energetic species including ions, electrons, and protons.	Produce oxygen and nitrogen species that are highly reactive (RONS)

The cytosol begins to shrink during the initial phase first bubbling begins, which also creates some holes. the tiny bubbles begin to reveal their larger size and release through the microscopic holes formed by cytosol aggregation. Compared to the earlier stage, the cytosol shrinks more. Then the bubbles begin to separate from the cell membrane. the nucleus components of the cell membrane have been destroyed, leading to cell death, and Detachment that how it leads to physically triggered cell death.	Chemical factors maintain pH in between extracellular tissues and the microenvironment of tumor tissue by acidifying extracellular tissue to encourage anti-cancer effects. (RONS) can modify the concentration of the amino acids tryptophan (Trp) and tyrosine (Tyr). These modifications can occur due to the ionization and oxidative reactions generated by the plasma.
The physical factor process is successful in reducing the size of melanoma tumors and preventing the creation of new tumors. This is accomplished by having antiproliferative and apoptotic effects on cancer cells, which leads to the significant elimination of cancer cells. resulting in the eventual death of the cells caused by physical triggers.	Using liquid Phosphate-buffered saline (PAPBS) chemical-based treatment leads to cell pathogen damage and helps to physical factors to induce apoptosis and necrosis but indirect plasma treatment cannot induce cell death directly.

2.5 Plasma categories

2.5.1 Oxidizing substances, including air, nitrogen oxide, dioxygen, and water

cold atmospheric plasma can be generated using oxidizing substances, including air, nitrogen oxide, dioxygen, and water. Cold atmospheric plasma refers to plasma that is produced at or near room temperature and atmospheric pressure.

In cold atmospheric plasma devices, a high-frequency AC or pulsed DC voltage is applied to electrodes, ionizing the gas and creating plasma. The oxidizing substances, such as air or dioxygen, provide the energy needed to sustain the plasma and generate reactive species, such as ions, radicals, and UV photons.

The properties of the plasma generated by cold atmospheric plasma devices can be influenced by various factors, such as the type of gas used, the voltage applied to the electrodes, the frequency of the voltage, and the pulse duration.

2.5.2 Reducing gases: hydrogen

Reducing gases, particularly hydrogen, can be used to create cold atmospheric plasma. Plasma created at or near room temperature and atmospheric pressure is referred to as cold atmospheric plasma. A high-frequency AC or pulsed DC voltage is supplied to electrodes in cold atmospheric plasma devices, ionizing the gas and producing plasma. The reducing gases, such as hydrogen, give the necessary energy to sustain the plasma and produce reactive species, such as ions, radicals, and UV photons (Yadav et al., 2020).

Various elements, such as the type of gas utilized, the voltage given to the electrodes, the frequency of the voltage, and the pulse duration, can influence the plasma characteristics formed by cold atmospheric plasma devices.

2.5.3 Gases containing nitrogen: ammonia

Nitrogen-containing gases, such as ammonia, can be used to produce cold atmospheric plasma. Cold atmospheric plasma refers to a plasma that is formed at or near ambient temperature and atmospheric pressure. High-frequency alternating current (AC) or pulsed direct current (DC) supplied to electrodes in cold atmospheric plasma devices ionizes the gas, forming a plasma. Nitrogen-containing gases, like ammonia, are the primary energy source for the plasma and the production of reactive species like ions, radicals, and UV photons.

The gas used, the voltage given to the electrodes, the frequency of the voltage, and the pulse duration are just a few of the variables that might affect the parameters of the plasma formed by cold atmospheric plasma devices.

2.5.4 Gases containing fluorine: sulfur hexafluoride and carbon tetrafluoride

Using fluorine-containing gases like sulfur hexafluoride and carbon tetrafluoride, cold atmospheric plasma can be produced. The term "cold atmospheric plasma" describes plasma that is created at or near ambient temperature and atmospheric pressure.

High-frequency alternating current (AC) or pulsed direct current (DC) supplied to electrodes in cold atmospheric plasma devices ionizes the gas, forming a plasma. Energy for the plasma and the production of reactive species including ions, radicals, and UV photons comes from fluorine-containing gases like sulfur hexafluoride and carbon tetrafluoride (Yadav et al., 2020).

2.5.5 Utilization of monomer gases, polymerizing gases can modify a layer upon a substrate or directly polymerize a layer

Atmospheric cold plasma can be produced by using monomer gases and polymerizing gases to either alter an existing layer on a substrate or polymerize a new layer from scratch. Plasma generated at or near atmospheric pressure and temperatures is known as "cold atmospheric plasma."

High-frequency alternating current (AC) or pulsed direct current (DC) supplied to electrodes in cold atmospheric plasma devices ionizes the gas, producing plasma. In order to sustain the plasma and produce reactive species like ions, radicals, and UV photons that can be exploited to alter the surface characteristics of materials or directly polymerize a layer, energy must be drawn from the monomer gases and polymerizing gases. The gas used, the voltage given to the electrodes, the frequency of the voltage, and the pulse duration are just a few of the variables that might affect the parameters of the plasma formed by cold atmospheric plasma devices (Yadav et al., 2020).

Atmospheric plasma gases of various types show up in a variety of contexts, most notably surface modification. For instance, organic components can be removed or left as surface oxygen species by using oxidizing gases. As a result of oxidation, surfaces become contaminated as reducing gases like fluorine or oxygen take their place. But nitrogen-containing gases are used to create an amino group on the substrate's surface, which improves the substrate's biocompatibility, wettability, and bondability. While fluorine-containing gases allow for plasma polymerization and etching, creating surfaces that are inert and hydrophobic, respectively.

we discussed various in vivo data as well as the cellular responses to therapy with cold atmospheric plasma for its anti-cancer effects.

Chapter 3

Plasma generating equipment

3.1 Generation of plasma using a vacuum pump

Plasma can be generated in a vacuum using a vacuum pump. This process is known as vacuum plasma generation or vacuum arc discharge. In vacuum plasma generation, a vacuum pump is used to create a low-pressure environment, often on the order of a few millitorrs or less. This low-pressure environment can then be utilized for a variety of purposes.

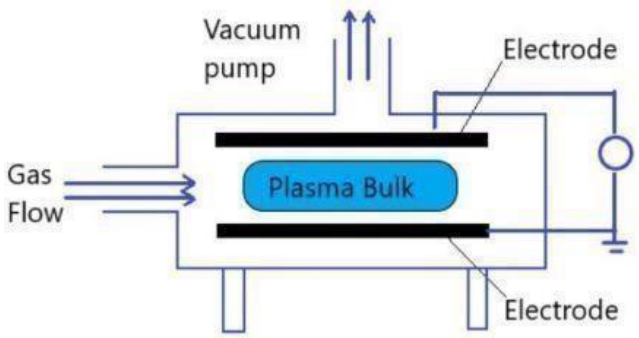

Figure 1: Generation of Plasma using a vacuum pump (Vesel, 2021).

An electric current is then applied to the plasma-generating device, which typically consists of an anode and a cathode. The electric current ionizes the gas in the vacuum, creating a plasma. Vacuum plasma generation is used in a number of applications, including the production of thin

films, the synthesis of nanomaterials, and the production of high-brightness plasma sources for spectroscopy and analytical applications. Vacuum plasma generation is also used in many industrial applications, such as plasma cutting, plasma welding, and plasma spray coating. In these

applications, plasma is used to modify the surface properties of materials, such as metals and polymers (Vesel, 2021).

In conclusion, plasma can be generated in a vacuum using a vacuum pump, a process known as vacuum plasma generation or vacuum arc discharge. Vacuum plasma generation is used in a wide range of applications, including the production of thin films, the synthesis of nanomaterials, and the production of high-brightness plasma sources for spectroscopy and analytical applications (Vesel, 2021).

Classification of plasma-inducing technology procedures is microwave-induced plasmas, corona discharges, dielectric barrier discharges, atmospheric pressure plasma jets.

3.2 Microwave-induced plasmas

Microwave-induced plasma systems are used to generate plasma through the interaction of microwave energy with a gas. In this process, a microwave generator produces high-frequency electromagnetic radiation, which is directed into a chamber containing a gas. The microwave energy excites the gas molecules, ionizing them and creating a plasma.

Microwave-induced plasmas are used in a variety of applications, including atomic emission spectroscopy, mass spectrometry, plasma etching, and plasma deposition. In these applications, plasma is used to modify the surface properties of materials, such as metals, polymers, and semiconductors (Fujimura, 2020).

In some microwave-induced plasma systems, microwave energy is coupled to the plasma through an antenna. In others, the plasma is generated within a waveguide, which is a hollow metal tube that guides the microwave energy into the plasma. The waveguide is typically coated with a

dielectric material, which helps to maintain the microwave energy within the waveguide and prevent it from escaping.

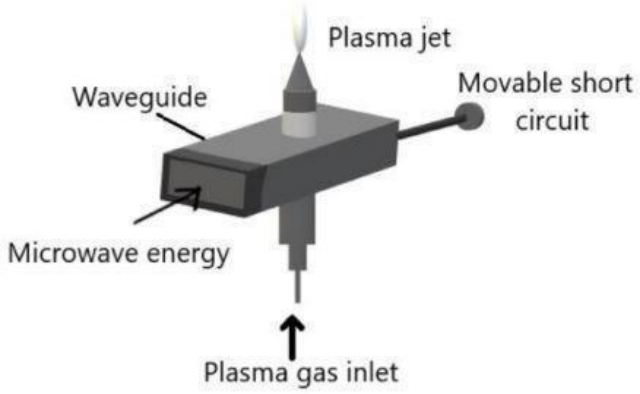

Figure 2: Microwave-induced plasmas device. (Fujimura, 2020)

The properties of the plasma generated by a microwave-induced plasma system can be controlled by adjusting various parameters, such as the pressure, temperature, and composition of the gas, the frequency and power of the microwave energy, and the design of the waveguide or antenna.

In conclusion, a microwave-induced plasma system generates plasma through the interaction of microwave energy with a gas. Microwave-induced plasmas are used in a variety of applications, including atomic emission spectroscopy, mass spectrometry, plasma etching, and plasma deposition. The properties of the plasma can be controlled by adjusting various parameters, such as the pressure, temperature, and composition of the gas, the frequency and power of themicrowave energy, and the design of the waveguide or antenna (Fujimura, 2020).

3.3 Corona discharge

Corona discharges are a type of electrical discharge that generates plasma. They occur when a high electrical potential is applied to a conductor, causing the electrical field near the conductor to become strong enough to ionize the surrounding air or other gas, creating a plasma.

Corona discharges can be classified into two types: positive and negative. Positive corona discharges occur when the electrical potential is applied to a positively charged conductor, while negative corona discharges occur when the electrical potential is applied to a negatively charged conductor (Fujimura, 2020).

Corona discharges are commonly observed in high-voltage electrical equipment, such as power transformers, high-voltage cables, and electric motors. They can also be generated in laboratory settings using high-voltage power supplies.

The properties of the corona discharge plasma can be influenced by various factors, including the voltage applied to the conductor, the size and shape of the conductor, the distance between the conductor and the surrounding gas, and the temperature and pressure of the gas (Wakamatsu, 2020).

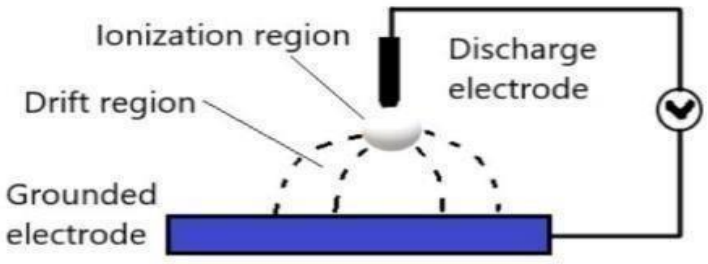

Figure 3: Corona discharges method (Wakamatsu, 2020).

In conclusion, corona discharges are a type of electrical discharge that generate plasma by ionizing the surrounding air or other gas. They can be classified into positive and negative corona discharges, and their properties can be influenced by various factors, such as the voltage applied to the conductor, the size and shape of the conductor, and the temperature and pressure of the surrounding gas.

Corona discharge has various uses, including the elimination of toxic volatile organic compounds from the environment, material surface treatment, gaseous pollution treatment, water purification, and ozone production, due to its many advantages, including its simplicity (Wakamatsu, 2020).

3.4 Dielectric Barrier Discharges

Dielectric barrier discharges systems are used to produce plasma. DBD is a type of electrical discharge that occurs between two electrodes separated by a dielectric material, such as glass or plastic. In a DBD system, an alternating voltage is applied to the electrodes, creating an electrical field that ionizes the gas between the electrodes, generating plasma. The dielectric material helps to prevent the electrical discharge from reaching the electrodes, allowing the plasma to be maintained at a high voltage while avoiding an electrical breakdown. DBD systems are used in a variety of applications, including surface treatment, air purification, and plasma chemistry. In these applications, plasma is used to modify the surface properties of materials, such as metals and polymers, or to generate reactive species for chemical reactions (Yan et al., 2012).

The properties of the plasma generated by a DBD system can be influenced by various factors, such as the voltage applied to the electrodes, the pressure and composition of the gas, and the design of the dielectric material (Pathania, 2022).

In conclusion, Dielectric barrier discharges systems produce plasma by ionizing the gas between two electrodes separated by a dielectric material. DBD systems are used in a variety of applications, including surface treatment, air purification, and plasma chemistry, and the properties of the plasma can be influenced by various factors, such as the voltage applied to the electrodes, the pressure and composition of the gas, and the design of the dielectric material (Pathania, 2022).

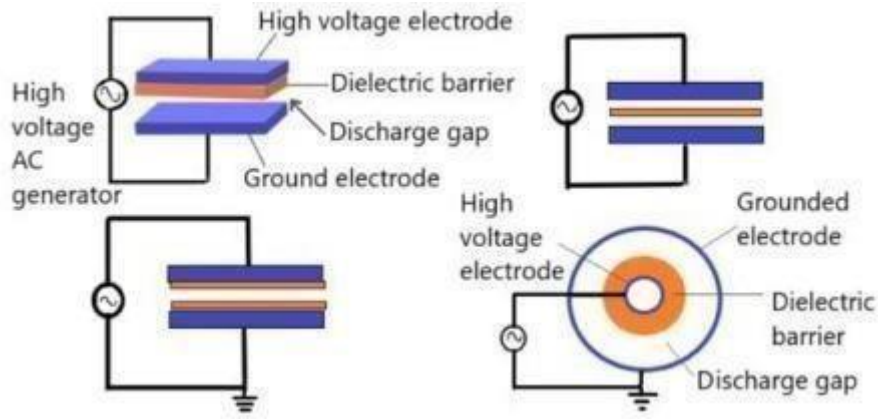

Figure 4: Dielectric barrier discharges method (Pathania, 2022).

The dielectric barrier discharge consumes less energy, has reduced operational costs, is scalable, is effective, and processes data quickly compared to corona discharges. While the principal drawbacks—high ignition voltage and a narrow discharge gap height—are connected with plasma homogeneity, they are nonetheless significant. Based on its advantages, dielectric barrier discharge plasma has a variety of applications, including the creation of ozone, the activation of CO lasers and excimer lamps, the treatment of wastewater, plasma chemical vapor deposition, and surface changes. Plasma generated by dielectric barrier discharge has substantial antibacterial properties, which is why it is used to improve food safety and extend food shelf life. Additionally, dielectric barrier discharge has been utilized well in a range of medical sectors, including regenerative medicine, dentistry, and cancer treatment (Pathania, 2022).

3.5 Atmospheric Pressure Plasma Jets

The atmospheric plasma jet (APPJ) devices generate plasma at atmospheric pressure, without the need for vacuum conditions. They are designed to ionize a gas, typically air or nitrogen, at or near atmospheric pressure, creating a plasma. APP devices typically use an electrical discharge to ionize the gas, which is maintained by a power supply connected to electrodes. The discharge is usually created by applying a high voltage to the electrodes, either by applying a high-frequency AC voltage or a pulsed DC voltage (Yan et al., 2020). The properties of the plasma generated by APP devices can be influenced by various factors, such as the type of gas used, the voltage applied to the electrodes, the frequency of the voltage, the pulse duration, and the distance between the electrodes. APP devices are used in a variety of applications, including surface treatment, air purification, and plasma-assisted chemical reactions. In these applications, plasma is used to modify the surface properties of materials, such as metals and polymers, or to generate reactive species for chemical reactions (Yan et al., 2012).

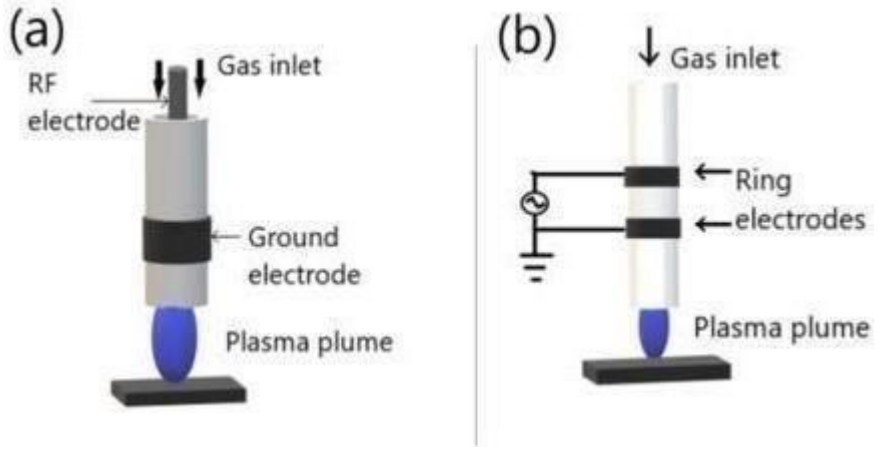

Figure 5: Atmospheric pressure plasma jets device (Yan et al., 2020).

In conclusion, Atmospheric Pressure Plasma (APP) devices produce plasma by ionizing a gas, typically air or nitrogen, at atmospheric pressure, using an electrical discharge. The discharge is typically created by applying a high voltage to electrodes, either by using a high-frequency AC voltage or a pulsed DC voltage, and the properties of the plasma can be influenced by various factors, such as the type of gas used, the voltage applied to the electrodes, and the distance between the electrodes. APP devices are used in a variety of applications, including surface treatment, air purification, and plasma-assisted chemical reactions.

Chapter 4

Application of CAP

4.1 Plasma vaccination in vivo experiment

B16F10 cancer cells are injected into xenografted tumor models (mice) to develop melanoma. Immunizing infected mice with a plasma-treated cell vaccination and observing them for 21 days. The efficacy of the plasma-treated immunization is compared to that of mitomycin (MMC) and mitoxantrone in the treatment of melanoma (MTX). Mitomycin (MMC) corresponds to a low immune profile concentration, whereas mitoxantrone (MTX) corresponds to a high immune profile concentration.

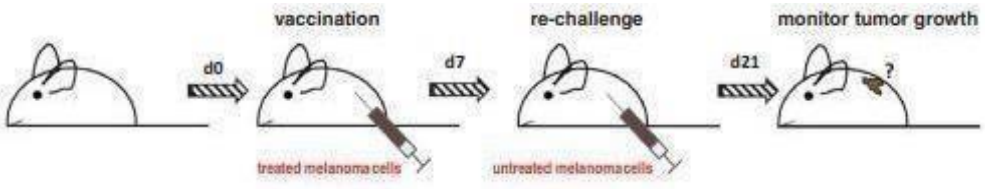

Figure 6: Monitoring tumor growth in xenograft model (Pathania, 2022).

After vaccination, changes in tumor cell proliferation were found in mice. Highly concentrated deposition of reactive oxygen species and nitrogen species (RONS) without heat sensation in the target tissue. One of the largest factors of hormone-acting chemicals that, at high doses, increase intracellular toxicity and, at low amounts, signal. On the other hand, the species that are typically created by gas plasmas are not ones that directly affect the cells or tissues of the body. ROS formed by plasma is more likely to have biological effects. It may have an effect on the immune system.

It might change the concentration of the immune system. The plasma vaccine suppresses cancer cells and shrinks their size.

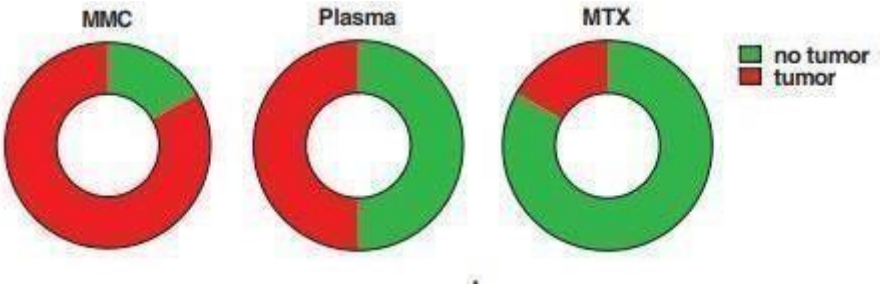

Figure 7: Comparison of immunological profile among plasma (Pathania, 2022).

Mitomycin (MMC) denotes a low concentration of the immunological profile, whereas mitoxantrone denotes a high concentration (MTX). The patterns in terms of immune infiltration and the activation of T cells observed in syngeneic melanomas in mice treated with plasma jet were enhanced. Melanoma cells were treated in vitro with either argon gas plasma or medicines with a known low (mitomycin C, MMC) or high (mitoxantrone) immunogenic profile in order to determine the influence that gas plasma therapy had on immunogenicity. Evaluation of the metabolic activity of melanoma cells demonstrated the toxicity of the drugs. In order to determine the ICD-nature of the medications and plasma therapy, levels of the anti-phagocytic molecule CD47, the eat-me signal calreticulin (CRT), the ICD marker heat-shock protein 90 (HSP90), and The major histocompatibility complex MHCI were measured using multicolor flow cytometry after an incubation period of 24 hours. During plasma therapy and MTX treatment, CD47 levels increased significantly, which may indicate phagocytosis sample reduction.

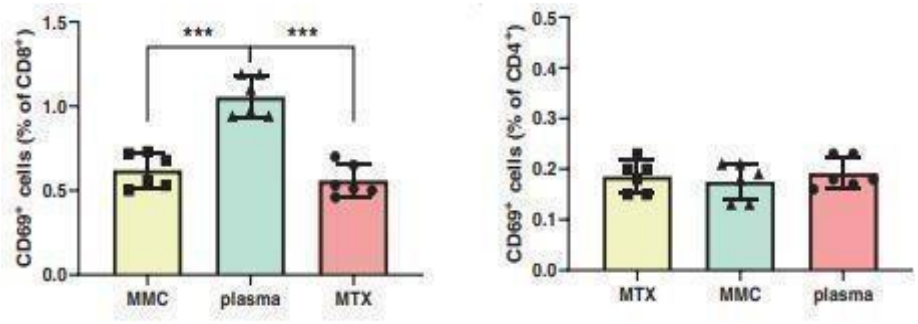

Figure 8: Comparison of immunological profile concentration among plasma (Pathania, 2022).

The nuclear translocation of the nuclear factors of activated T-cells (NFAT), nuclear factor E2-related factor 2 (Nrf2), and nuclear factor "kappa-light-chain-enhancer" of activated B-cells (NFB) in relation to plasma and drug exposure was investigated by using quantitative high-content image analysis. The ratio of the nuclear mean absorbance value to the cytoplasmic mean absorbance value was determined as a way to evaluate the nuclear translocation and gene activation of each transcription factor (Yadav et al., 2020).

During the plasma vaccination experiment, mice that already have formed xenografted tumors are subjected to cold atmospheric plasma (CAP), which is produced from a gas combination consisting of elements including helium, argon, and nitrogen. Exposure to CAP causes the tumor cells to sustain damage, which ultimately leads to the release of tumor antigens. Following this, the antigens are picked up by dendritic cells, which then deliver them to the immune system. This results in the activation of T cells that are specific to the tumor.

After this, the activated T cells travel to the location of the tumor, where they assault the cancer cells, which ultimately results in a reduction in the growth of the tumor. It has been demonstrated that vaccination with plasma can in some cases bring about total regression of the tumors.

In conclusion, in vivo investigations with xenografted tumor models in mice employing plasma vaccination have showed promising results as a technique for activating an immune response against malignancies. When tumor cells are subjected to cold atmospheric plasma, this causes the release of tumor antigens, which are then taken up by dendritic cells and presented to the immune system. This results in the activation of tumor-specific T cells, which in turn leads to a reduction in the growth of the tumor (Yadav et al., 2020).

4.2 Extracellular tissues are acidified to induce anti-cancer effects

Because of metabolic changes, the microenvironment around intracellular tumors is frequently acidic. Due to the increase in the rate of glycolysis as well as the pentose phosphate pathway, as well as inadequate oxygen delivery compared to metabolic demand. Although the pH of the extracellular environment of most tissues is approximately 7.4, the pH of the microenvironment of a tumor can frequently range from 5.4 to 7.4. The epidermoid carcinoma cell line is unaffected by variation in the extracellular pH of 6, but skin cells like fibroblasts or endothelial cells, as well as neuronal cells, are all impacted. In melanoma cells, Ca2+ influx that was produced by cold atmospheric plasma was shown to be stronger at an acidic pH than it was under normal conditions.

This finding suggests a synergistic process between acidification and cold atmospheric plasmainduced species. After exposure to cold atmospheric plasma, we saw that solutions became more acidic. Cold atmospheric plasma's (CAP) anticancer properties are affected by H_2O_2, hydroperoxyl HO_2, or proximations acid to induce acidification (Wang et al., 2017).

Nitrous acid (HNO_2) and nitric acid (HNO_3) are produced by the hydrolysis of nitrogen dioxide (NO_2). HNO_3 is a strong acid; the pKa value of HNO_3 is 1.32, and it completely dissociates in water. HNO_2 is a weak acid. The pKa value of HNO_2 is 3.3; that only dissociates in a very limited

manner, and it is in a state of acid-base equilibrium with nitrite (NO_2). NaN3 has the ability to reduce the acidification induced by cold atmospheric plasma in aqueous solutions. This outcome was attributed to a NO_2 response. use NaN3, which absorbs protons. The actual mechanism confirmed our assumption that HNO_2 might also be contributing to the acidification caused by the cold atmospheric plasma (Woedtke et al., 2020).

Two moles of NO_2 contribute to the acidification of water. Nitrous acid (HNO_2) and nitric acid (HNO_3) are produced by the hydrolysis of nitrogen dioxide (NO_2). Strong acid hydrolysis causes the dehydrogenation of HNO_3 to produce NO_3. Dehydrogenation from HNO_2 occurs during the hydrolysis of weak acids, producing HNO_2 (Wang et al., 2017).

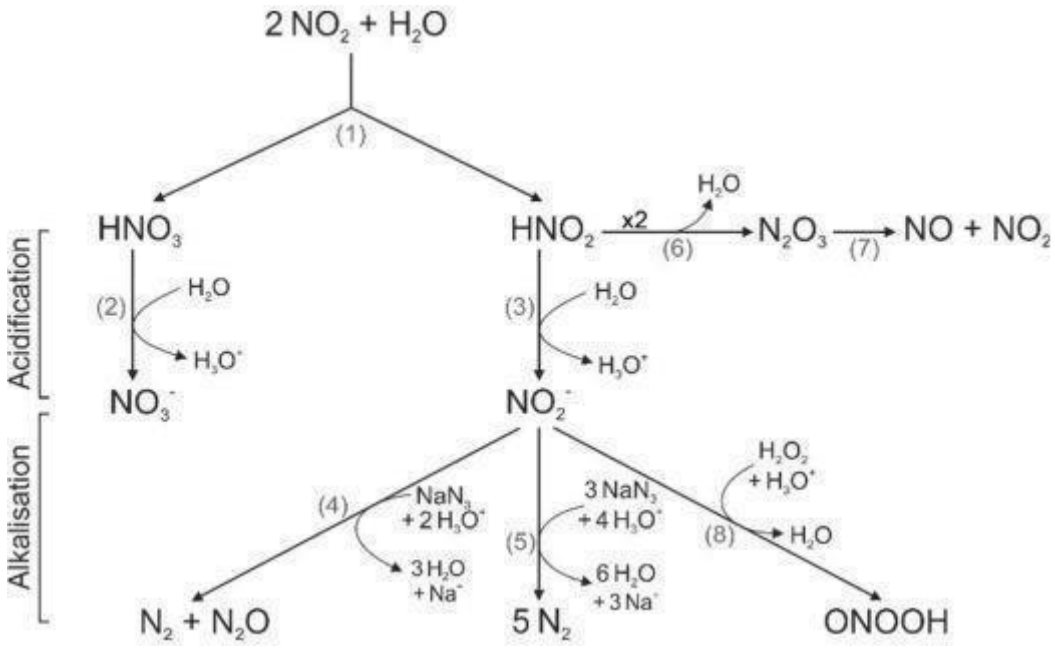

Figure 9: Extracellular tissues acidification. (Woedtke et al., 2020)

the acidification caused by CAP was crucial to the rise of intracellular Ca^{2+} that CAP caused in melanoma cells. Stronger buffer systems inhibited acidification, which in turn lowered the CAP-induced Ca^{2+} influx. It generates HNO_3 and HNO_2, both of which are toxic.

As we showed that the nitration of proteins and generation of NO that is CAP-induced was pH-dependent, we hypothesize that hydrolysis of CAP-induced NO_2 is responsible for this acidification. Scavenger tests demonstrated that ROS played a similar role to that of RNS in CAP-induced Ca^{2+} influx, which indicates a synergistic effect of ROS, RNS, and acidic conditions. Even while this will only assist in the development of CAP as a potential cancer therapy, it is crucial to recognize that CAP generates a complicated mixture of unknown formulations. In some cases, such as following tumor surgery, CAP therapy may be administered. There is a high probability that the acidic environment of tumors helps the CAP actions succeed and contributes to the cancer cell inhibiting activities.

The pH of the microenvironment and extracellular space of tumor cells is a critical factor that affects the outcome of various cancer treatments, including the treatment of melanoma using cold atmospheric plasma (CAP). The pH level of these environments plays a crucial role in regulating cellular processes, including cell survival, proliferation, and apoptosis, which are critical components in the effectiveness of cancer treatments. The maintenance of a specific pH level is essential in ensuring that the treatment is as effective as possible and that it targets the cancer cells while minimizing damage to normal, healthy cells (Woedtke et al., 2020).

In melanoma treatment with CAP, the pH level is crucial because CAP generates reactive species, such as radicals, that are toxic to cancer cells. These reactive species are generated by the plasma-induced ionization of the surrounding gases, which results in the production of ions, electrons, and

reactive species such as hydrogen peroxide, superoxide, and hydroxyl radicals. These reactive species have the ability to damage cellular components, including DNA, proteins, and lipids, leading to cell death (Yadav et al., 2020).

However, changes in the pH of the microenvironment can affect the production and stability of these reactive species, and thus, their ability to kill cancer cells. For example, an acidic environment can lead to the increased production of radicals composed of hydroxyl, which are highly reactive and toxic to cells. On the other hand, an alkaline environment can reduce the production of reactive species, leading to decreased effectiveness of the treatment. This highlights the importance of maintaining the pH of the microenvironment at a specific level to ensure optimal efficacy of CAP in treating melanoma.

In addition to affecting the production of reactive species, changes in the pH level of the microenvironment can also impact the activity of various enzymes and metabolic pathways involved in cancer cell survival and death. For instance, cancer cells often have altered pH regulation mechanisms compared to normal cells, which can result in the creation of an acidic microenvironment that promotes cell survival and inhibits cell death. By maintaining the pH at a specific level, the activity of these enzymes and metabolic pathways can be modulated, leading to increased cell death and decreased cell survival (Yadav et al., 2020).

Furthermore, the pH of the extracellular space also plays a critical role in the effectiveness of CAP treatment. The extracellular space is a critical component in the transport of ions and molecules, including nutrients and waste products, between cells. A change in the pH of this environment can impact the transport of these critical components, leading to changes in cellular metabolism and

cellular processes. This highlights the importance of maintaining the pH of the extracellular space at a specific level to ensure the optimal effectiveness of CAP in treating melanoma.

In conclusion, the pH of the microenvironment of tumor cells and the extracellular space is a critical factor that affects the effectiveness of melanoma treatment using cold atmospheric plasma. Maintaining the pH at a specific level is essential in ensuring the optimal production and stability of reactive species, as well as modulating cellular processes involved in cell survival and death. These factors all contribute to the overall efficacy of CAP in treating melanoma, making the maintenance of pH levels a critical aspect of this treatment. The intracellular Ca^{2+} homeostasis of melanoma cells is affected by a combined combination of ROS, RNS, and acidic conditions caused by CAP. One possible explanation for CAP's targeted anti-cancer effects is that it neutralizes the acidic microenvironment that commonly envelopes tumors.

4.3 Cold plasma causes the biological death of cancer cell

Cold atmospheric plasma is a partly ionized gas that causes reactive oxygen and nitrogen species. Long-lived reactive species, such as reactive oxygen species (ROS), and short-lived reactive species, such as reactive nitrogen species, contribute to ecological degradation (RNS) Plasma in the cold atmosphere also generates physical factors. It emits heat, has an electromagnetic (EM) effect, and generates infrared energy. Due to its multimodal chemical and physical nature, cold plasma is a suitable, controlled, adaptable, and even self-adaptive instrument for the treatment of dermatitis wound healing, and cancer therapy.

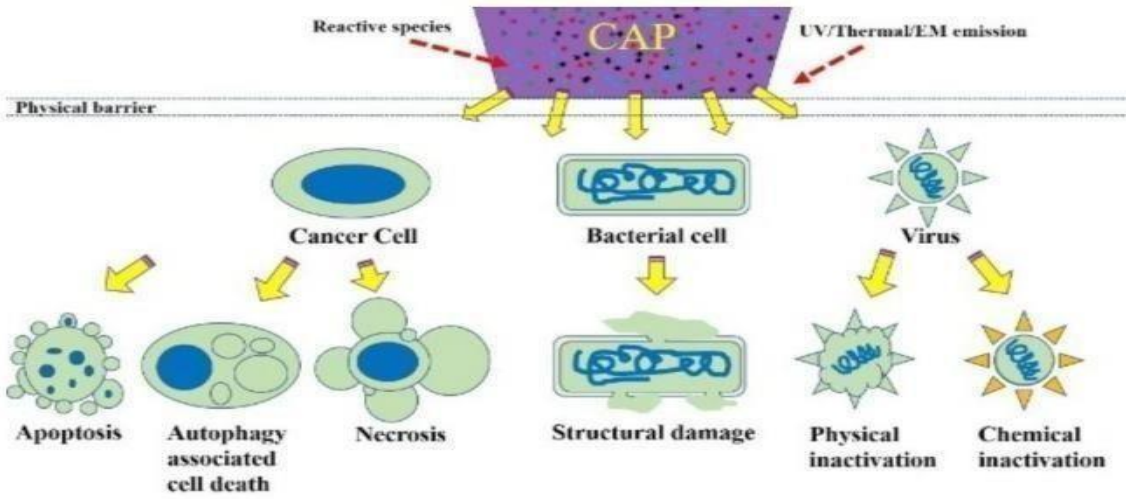

Figure 10: Process of killing of cancer cells by the biological effects of cold plasma (Yadav et al., 2020).

Cold atmospheric plasma can cause strong damage to both gram-positive and gram-negative bacteria. The various bacterial species that make up the biofilm's population can be successfully eradicated if cold atmospheric plasma therapy is utilized. A significant improvement in the efficiency of wound healing is achieved. Scientific studies in vitro and in vivo over the course of the last decade have shown that cold plasma has significant potential as a novel cancer treatment. Cold plasma offers tremendous potential as an anticancer therapy.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) will create oxidative stress in cells that have been exposed to cold atmospheric plasma, which will trigger cell-death pathways if the reactive species dose on a single cancer cell is high enough. It has been speculated for a long time that physical factors like heat radiation and electromagnetic waves have biological effects, but until recently there was no clear evidence of this. A possible reason is the electromagnetic influence of the cold plasma jet, which rapidly produces necrosis and causes structural damage to melanoma cells.

The mixed bacterial community that makes up the biofilm can similarly be effectively inactivated using cold atmospheric plasma therapy. significantly increase the effectiveness of wound healing. In the past 10 years, research conducted in vitro and in vivo on cancer patients has revealed that cold plasma may have significant promise as a novel anti-cancer therapy. If the reactive species dose on a single cancer cell is high enough, reactive oxygen species (ROS) and reactive nitrogen species (RNS) will generate oxidative stress in cells exposed to cold ambient plasma and activate cell-death pathways. It has been speculated for a long time that physical factors like heat radiation and electromagnetic waves have biological effects, but until recently there was no clear evidence of this. A possible reason is the electromagnetic influence of the cold plasma jet, which rapidly produces necrosis and causes structural damage to melanoma cells.

When compared to indirect cold atmospheric plasma treatment, which is based on CAP-treated fluids including medium, phosphate-buffered saline (PBS), and water, direct cold atmospheric plasma treatment, which is based on physical and chemical processes, causes more harm to both cells and pathogens. The impacts of long-lived reactive species, short-lived reactive species, and physical parameters are all implicated in the direct cold atmospheric plasma treatment. In contrast to this, indirect therapy utilizing a cold atmospheric plasma will only have a biological effect due to the presence of reactive species with prolonged half-lives. For cytotoxicity to occur against cancer cells, long-lived reactive species are required. However, long-lived reactive species are not able to precisely explain the anti-cancer activity that has been demonstrated. It's possible that short-lived reactive species can change the biological responses of cancer cells, turning them into more effective cytotoxic targets for direct treatment rather than indirect treatment (Yadav et al., 2020).

On the one hand, plasma-treated cancer cells have demonstrated a high micromolecular H2O2 generation. In contrast, cancer cells immediately exposed to CAP undergo activation and become

more vulnerable to the cytotoxicity of long-lived reactive species, such as hydrogen peroxide (HNO_2) and nitrogen dioxide (NO_2).

Reactive species of cold plasma, including intracellular ones, induce apoptosis. Reactive oxygen species are the principal cause of cell death (ROS). Supplementation with intracellular ROS antioxidants, such as N-acetylcysteine (NAC), can effectively interfere with cancer cell-TME interactions by reducing the ROS signaling that is mediated by NAC and protecting cells from the cytotoxicity of cold atmospheric plasma therapy.

4.4 Physically triggered cancer cell death by trans barrier cold Plasma

Cold atmospheric plasma increases electromagnetic waves and chemical compounds such as reactive species into cancer cells, hence eliminating the physical barrier and triggering the process of cell death.

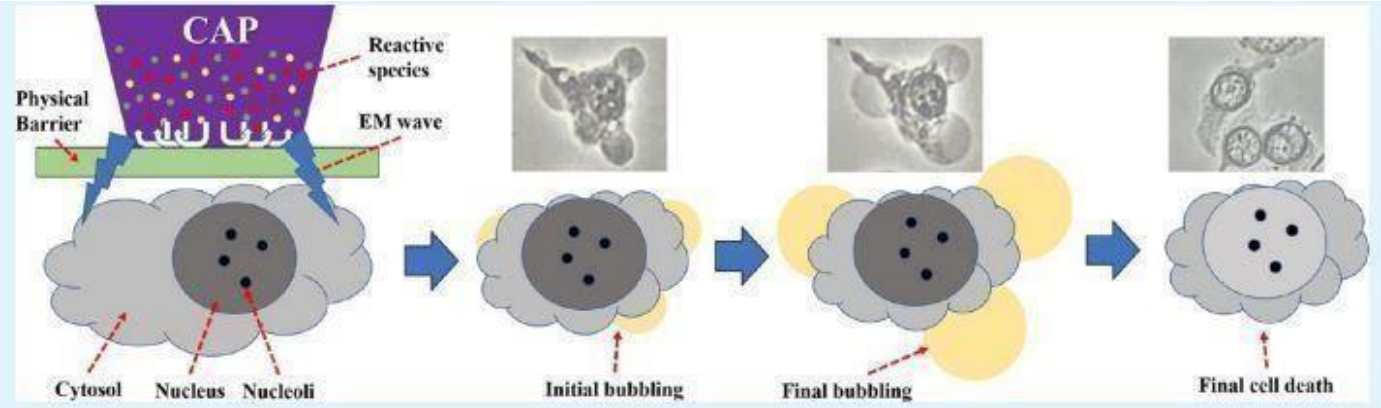

Figure 11: Trans barrier cold plasma physically triggers cancer cell death (von Woedtke et al., 2013).

This research illustrates how treatment with cold atmospheric plasma can lead to considerable cell death. through a manner that is not direct and does not involve physical touch while passing

through a considerable (greater than 1 mm) physical barrier. When B16F10 cells are subjected to cold atmospheric plasma, the cytoplasm within the cells constricts and bubbles form on the cell membrane (von Woedtke et al., 2013).

Melanoma cells are susceptible to being killed by electromagnetic radiation that is emitted from cold atmospheric plasma using a contactless trans barrier approach. The B16F10 reactive species resistant melanoma cell line exhibits noticeably elevated levels of growth inhibition as a consequence of the physical factors. A newly discovered category of cell death known as physicallytriggered growth inhibition is responsible for the phenomenon. This type of cell death is characterized by rapid loss of bulk solution from cells, cytoplasmic contraction, and membrane bubbling. Even in the presence of a macroscale gap, it is possible for cells and cold atmospheric plasma to experience cell death when there is an 8 mm air gap between them and the dielectric substance of the dish or plate (1 mm). This type of cell death can be prevented by either having an excessively large or inadequate amount of space. In an environment that is hypotonic, the extracellular osmotic pressure prevents cells from bubbling as a result of the physically induced cellular pressure. This is because external osmotic pressure is higher than intracellular osmotic pressure (Turkoglu Sasmazel et al., 2021).

A cell line that is resistant to reactive oxygen species (ROS) or reactive nitrogen species (RNS) will not be affected by cold air plasma. These two types of reactive species are abbreviated as ROS and RNS, respectively. Melanoma cells with the B16F10 line are resistant to reactive species, in particular ROS such as hydrogen peroxide. investigated the significance of chemical and physical cold plasma treatments in the atmosphere as potential growth inhibitors and analyzed the results. Individuals who make it through a chemical cold atmospheric plasma therapy unharmed are the only ones who are able to suffer growth inhibition. In contrast, the cold Atmospheric Plasma

treatment suppresses the growth of melanoma cells in at least eight of the wells that are representative of the treated well (von Woedtke et al., 2013).

There are a total of four steps involved in the physical treatment procedure using cold atmospheric plasma. the entire process of cell death that is caused by physical triggers:

1. Cytosol aggregation 2. Bubbling 3. Detachment of bubbles 4. Post-bubbling events

The first two stages are short-lived cellular reactions that occur for an average number of about 10 minutes. The duration of bubble detachment can approach 12 hours. The post-bubbling occurrences, however, could continue for days. The cells may participate in the bubbling process. rapidly lose their moisture content. After the first step, the primary cytosol shape will not alter, giving the cells the appearance of being fixed following the procedure. Up to the final cell death or detachment, this "fix form" will be maintained for a period of days. Cytosol aggregation where the cytosol begins to shrink during the first 1-2 minutes of the process, and after a short while, the first bubbling begins, which also creates some holes. Bubbling after around 8 minutes, the tiny bubbles begin to reveal their larger size and release through the microscopic holes formed by cytosol aggregation. Compared to the earlier stage, the cytosol shrinks more. Detachment of bubbles about two hours later, the bubbles begin to separate from the cell membrane. Post-bubble events after a few days, the nucleus components of the cell membrane have been destroyed, leading to cell death and detachment (von Woedtke et al., 2013).

4.5 CAP Influences Solution Amino Acids and Melanoma Cell Anti-Tumor Effect

Although plasma-treated solutions (PTS) have less potent anticancer effects than direct cell treatment, their application in the treatment of malignant cells has been increasing. Injectable

plasma therapy, on the other hand, may be able to more precisely target cancer cells within the body than direct plasma therapy.

inside the area of the tumor. Cell culture mediums, which typically include a great deal of free amino acids, are the usual solutions utilized for the production of PTS. It is potential that the CAP-induced generation of RONS in the medium will be converted into other, longer-lived species, such as nitrite (NO_2) and nitrate (NO_3) or hydrogen peroxide. This is because these species have a higher rate of oxidation (H_2O_2). Hydrogen peroxide (H_2O_2), nitric oxide (NO_2), and nitric oxide are the three key active components that have been identified as being responsible for the anti-tumor activities exhibited by PTS (NO_3).

According to the results obtained the formation of reactive oxygen and nitrogen species (RONS), as well as the degradation and modification of the amino acids tyrosine (Tyr), and tryptophan, varied depending on the mode of action and the dose that was administered. This was one of the key takeaways from the study. These discoveries were outlined in the form of a series of conclusions that were given (Trp). The 4-kHz frequency was responsible for the formation of the Trp metabolites formyl kynurenine (FKyn) and kynurenine (Kyn) in the PTS (oxygen) pathway, which facilitated the activation of apoptosis in Mel Im melanoma cells. This resulted in the Mel Im melanoma cells being more susceptible to apoptosis. During the 8 kHz (nitrogen) mode, nitrated derivatives of Trp and Tyr were created, which led to a rise in the level of p16 mRNA expression as well as senescence-associated ß-Galactosidase staining. This was the case because nitrated derivatives of Trp and Tyr were formed. In conclusion, the plasma mode has a significant impact on the make-up of the active components that are present in PTS, which in turn has an impact on the manner in which it fights cancer. This is due to the fact that the mode of the plasma

decides what the composition of the active components will be. These findings are of the utmost significance for the advancement of plasma-based devices as well as the efficacy of cancer treatment (Soni et al., 2021).

Table 4: CAP impacts on PTS and melanoma cell molecular and biological pathways (Soni et al., 2021).

CAP Treatment Mode	Plasma- treated Solution (PTS)	4 kHz (Oxygen Mode) 5 min Duration	8 kHz (Nitrogen Mode) 5 min Duration
"RONS" production	"Dulbecco modified eagle's medium" + "fetal bovine serum" (DMEM+FBS)	ROS ++, H2O2 ++	NO2 ++, NO3 ++
	"Dulbecco modified eagle's medium" – "fetal bovine serum" (DMEM–FBS)	ROS +++, H2O2 +++	NO2 +++, NO3 +++
	tryptophan (Trp)	ROS ++, H2O2 +++	NO2 +++, NO3 ++
	Tyrosine (Tyr)	+ ROS ++, H2O2 —	NO2 +++, NO3 +++
"Amino acid" degradation	"Dulbecco modified eagle's medium" + "fetal bovine serum" (DMEM+FBS)	Tyr, Trp	Tyr
	"Dulbecco modified eagle's medium" – "fetal bovine serum"(DMEM–FBS)	Cys,His,Met, Phe, Tyr, Trp	Tyr, Trp
	tryptophan (Trp)	Trp	Trp
	Tyrosine (Tyr)	Tyr	Tyr
"Amino-acid" modification	"Dulbecco modified eagle's medium" – "fetal bovine serum"(DMEM–FBS)	HTyr, FKyn, Kyn	NTyr, NTrp
	tryptophan (Trp)	HTrp, FKyn, Kyn	NTrp
	Tyrosine (Tyr)	NTyr	NTyr
Coloration of PTS	"Dulbecco modified eagle's medium" + "fetal bovine serum" (DMEM+FBS)	+ +	+ +
		+ +	

Coloration of PTS	"Dulbecco modified eagle's medium" – "fetal bovine serum"(DMEM–FBS)		
		+	
	tryptophan (Trp)	+	
	Tyrosine (Tyr)		
p16 gene expression	all Plasma- treated Solution (PTS)	+	+ + +
Induction of apoptosis	(Dulbecco modified eagle's medium) + (fetal bovine serum) (DMEM+FBS)	++	
	(Dulbecco modified eagle's medium) – (fetal bovine serum)(DMEM–FBS)	+++	
	tryptophan (Trp)	+++	
	Tyrosine (Tyr)		
Induction of senescence	(Dulbecco modified eagle's medium) + (fetal bovine serum) (DMEM+FBS)	+	+ + +
	(Dulbecco modified eagle's medium) – (fetal bovine serum)(DMEM–FBS)	+	+ + +
	Tryptophan (Trp)	+	+ +
	Tyrosine (Tyr)		

cold atmospheric plasma (CAP) alters the amino acid content of solutions and modifies the anti-tumor action on melanoma cells. This is accomplished by generating reactive species, such as ions, electrons, and radicals, that interact with the components of cells. These interactions can result in alterations to the amino acid content of the solution as well as the environment of the cells, which can therefore have an effect on the anti-tumor activity. CAP treatment can cause the ionization of amino acids in the solution, leading to the formation of charged amino acids or peptides. These charged species can interact with the melanoma cells, causing changes in the amino acid composition and potentially leading to cell death. In addition, the reactive species generated by CAP, such as hydrogen peroxide and hydroxyl radicals, can directly interact with cellular components, including proteins and lipids, causing oxidative damage and leading to cell death.

Furthermore, the changes in the amino acid composition of the solution can also impact the function of proteins involved in cellular processes, including cell survival and death. For example, the oxidative damage caused by the reactive species generated by CAP can cause changes in the structure of proteins, leading to alterations in their function and potentially leading to cell death.

The treatment of solutions with cold atmospheric plasma (CAP) has the potential to result in changes to the amino acids tyrosine and tryptophan (Trp) and (Tyr). Ionization and oxidation are two types of processes that can be caused by plasma, and both can lead to the previous mentioned changes. Tryptophan is an aromatic amino acid that is sensitive to oxidative stress and can be easily modified by reactive species, such as hydroxyl radicals and hydrogen peroxide, generated by CAP. This can lead to the formation of oxidation products, such as indole derivatives and kynurenine, which can impact the biological activity of (Trp)-containing proteins and influence the anti-tumor effect on melanoma cells.

Tyrosine is another amino acid that is susceptible to oxidative modifications generated by CAP. Tyrosine can be oxidized to form a range of products, including tyrosyl radicals, Di tyrosine, and 3,4-dihydroxyphenylalanine (DOPA). These alterations have the potential to have an effect on the function of proteins that contain Tyr and could possibly contribute to the anti-tumor effect that CAP therapy has on melanoma cells. The effects of a five-minute treatment with 4 kHz and 8 kHz CAP on pure amino acids Trp and Tyr at a concentration of 1 mM in water were compared to the effects of an untreated control group using NMR spectroscopy (control). The amounts of both amino acids were far higher than anticipated, which was to be expected.

to result in a reduction in the overall and overall Tyr PTS In order to ascertain the matching reaction products, an investigation utilizing HPLC and TOFMS was carried out. High- Performance Liquid

Chromatography-Time of Flight Mass Spectrometry, or HPLC-TOFMS for short, is a very effective analytical method that can separate and characterize complicated mixtures. It combines the powers of separation that high-performance liquid chromatography (HPLC) possesses with the high-resolution detection and identification capabilities that time-of- flight mass spectrometry possesses (TOFMS) Nitro tryptophan (NTrp) was detected in the Trp solution CAP treated at 8 kHz for 5 minutes, but the rest of the observed chemicals, such as

Hydroxytryptophan (HTrp), Formyl kynurenine (FKyn), and Kynurenine (Kyn), were discovered in the Trp solution CAP treated at 4 kHz for 5 minutes. In addition, the CAP-treatment mode operating at 4 and 8 kHz was used, which resulted in a portion of the Tyr molecule being converted to nitro tyrosine (NTyr) (Turkoglu Sasmazel et al., 2021).

Treatment of fetal bovine serum (FBS) in Dulbecco's modified eagle medium (DMEM) with cold atmospheric plasma can result in amino acid changes (CAP). The DMEM-FBS solution contains components that can react with reactive species produced by the CAP treatment, such as ions, electrons, and radicals. Amino acids in solution may oxidize, ionize, or fragmentation a result of these interactions. Tryptophan (Trp) and tyrosine (Tyr) can be oxidized by CAP treatment, yielding oxidation products including indole derivatives and kynurenine for Trp and tyrosyl radicals, Di tyrosine, and 3,4-dihydroxyphenylalanine (DOPA) for Tyr. The anti-tumor effect of CAP therapy on melanoma cells can be affected by these alterations, which affect the biological activity of Trp and Tyr-containing proteins. Cold atmospheric plasma's (CAP) anti-tumor action on melanoma cells may be affected by amino acid changes that occur during DMEM-FBS treatment. The modifications that have been observed are the result of interactions between the reactive species that are created by CAP and the components of the solution (Turkoglu Sasmazel et al., 2021).

In conclusion, the treatment of solutions with cold atmospheric plasma (CAP) can result in modifications to the amino acids tryptophan (Trp) and tyrosine (Tyr). These modifications have the potential to influence the biological activity of proteins that contain these amino acids and can also affect the anti-tumor effect on melanoma cells. Ionization and oxidation reactions brought about by the plasma are responsible for these developments (Soni et al., 2021).

Chapter 5

Discussion

Cold atmospheric plasma (CAP) is a new and innovative treatment for melanoma that is being considered as an alternative to chemotherapy and tumor removal surgery. CAP is a non-thermal, non-invasive method of treating cancer that has been shown to be safe and effective for a variety of different cancer types, including melanoma. In the following paragraphs, we will discuss the advantages of CAP in comparison to chemotherapy and surgical excision of tumors, as well as the benefits of utilizing this innovative technology in the treatment of melanoma.

In comparison to chemotherapy and surgical removal of the tumor, CAP is significantly safer. This is one of the most significant advantages of CAP. Unlike chemotherapy, which can cause substantial side effects such nausea, vomiting, and hair loss, CAP is non-invasive and does not cause any significant side effects. This enables patients to undergo treatment for CAP with very little to no discomfort and without the necessity of being hospitalized at any point during the process. Additionally, because CAP is a non-thermal treatment, it does not destroy surrounding healthy tissue. This makes it a safer option for people who have melanoma.

The efficacy of CAP stands as an additional benefit of the treatment in comparison to that of chemotherapy and surgical removal of the tumor. It has been demonstrated that CAP is an effective treatment for a diverse spectrum of cancer forms, including melanoma. This occurs as a result of the fact that CAP generates an electric field around the cancer cells, which in turn causes the cells to become damaged and eventually pass away. This indicates that CAP is able to successfully destroy cancer cells without causing damage to the healthy tissue that is around the cancerous cells. As a result, CAP is an extremely effective treatment for melanoma.

CAP is a viable option to both chemotherapy and surgical removal of tumors, and it does not incur the high costs associated with any of these procedures. The treatment for CAP is often far more cost-effective than chemotherapy, and it is also less intrusive than surgery. As a result, patients can receive treatment in a shorter amount of time and with less time required for recovery. This may be of utmost significance for patients diagnosed with melanoma, as they may be required to undergo many treatment cycles over the duration of their illness.

The adaptability of CAP is an additional benefit that sets it apart from chemotherapy and surgical removal of tumors. Unlike chemotherapy, which is often only helpful for a restricted range of cancer types, CAP is capable of treating a variety of various cancer forms, including melanoma. In addition, CAP can be utilized in conjunction with other treatments, including as chemotherapy and surgery, to obtain superior outcomes. Because of its adaptability, chemotherapy adjuvant photodynamic therapy is an extremely appealing choice for people with melanoma who are looking at alternative treatment choices.

Chapter 6

Conclusion

In conclusion, cold atmospheric plasma offers an alternative treatment for people with melanoma that is both safe and successful in comparison to chemotherapy and the surgical excision of tumors. Patients who are looking for a method that is both safe and effective in the treatment of their cancer will find this treatment option to be one of the most desirable options available. Its non-invasive nature and absence of substantial side effects contribute to this. Additionally, its adaptability and cost-efficiency make it an intriguing alternative to traditional cancer treatments. Furthermore, its effectiveness in treating a wide range of cancer types makes it an interesting option for patients who are looking for alternative therapy alternatives.

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