1 **Plasma-Induced DNA Damage and Repair Mechanisms: Investigating Genetic**
2 **Impact and Cellular Response Pathways**

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7 ## Abstract

8 The interaction of plasma with biological systems has emerged as a transformative approach in
9 modern biomedicine, with applications spanning cancer therapy, wound healing, tissue
10 regeneration, and microbial decontamination. Among various plasma types, Cold Atmospheric
11 Plasma (CAP) has gained prominence due to its ability to generate reactive oxygen and nitrogen
12 species (RONS) at near-room temperatures, making it suitable for direct biological applications.
13 However, CAP can also cause DNA damage, which raises concerns about its potential genotoxic
14 effects. This paper provides a comprehensive overview of how CAP induces various types of
15 DNA lesions, such as single- and double-strand breaks, base modifications, and crosslinks,
16 through the action of RONS. It further delves into the complex cellular responses activated upon
17 such damage, including key DNA repair pathways like base excision repair (BER), nucleotide
18 excision repair (NER), and homologous recombination (HR). Understanding these mechanisms
19 is crucial for harnessing plasma's therapeutic potential while ensuring genomic safety in clinical
20 applications.

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24 **Keywords :** Cold Atmospheric Plasma (CAP), DNA Damage, Genotoxicity, Reactive Oxygen
25 and Nitrogen Species (RONS), DNA Repair Mechanisms, Biomedical Applications

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1. Introduction

Plasma, widely recognized as the fourth fundamental state of matter, consists of a dynamic and partially ionized gas containing free electrons, ions, neutral atoms, UV photons, and electromagnetic fields. Unlike solids, liquids, or gases, plasma exhibits unique physical and chemical behaviors, particularly in terms of energy transfer and reactivity. In recent decades, scientific and technological advancements have enabled the generation of cold atmospheric plasma (CAP), a non-thermal form of plasma that operates effectively at or near room temperature. This has opened up groundbreaking possibilities for directly applying plasma to living tissues without causing thermal damage (Fridman et al., 2008; Keidar, 2015).

The use of CAP in biomedicine is an emerging interdisciplinary field combining plasma physics, molecular biology, and clinical science. CAP is now actively being explored for multiple therapeutic and diagnostic purposes, including sterilization, wound healing, antimicrobial treatments, and notably, cancer therapy (Laroussi, 2005; Hori, 2017; Van Boxem et al., 2012). The primary reason for this versatility lies in the rich mixture of reactive oxygen species (ROS) and reactive nitrogen species (RNS) it generates. These reactive species, such as hydroxyl radicals (•OH), hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), nitric oxide (NO•), and peroxynitrite ($ONOO^-$) can interact strongly with cellular components, especially nucleic acids (Lu et al., 2016).

While the therapeutic potential of CAP has been demonstrated in eradicating pathogens and selectively inducing cell death in cancerous tissues (Clancy et al., 2020; Ahn et al., 2014), its interactions with DNA, the cell's fundamental genetic blueprint, raise significant biosafety concerns. DNA is particularly vulnerable to oxidative stress induced by CAP, which can result in a spectrum of structural and chemical lesions (Zimmermann et al., 2012). These range from single-strand breaks (SSBs) and double-strand breaks (DSBs) to base modifications, crosslinks, and abasic sites, all of which can compromise genetic integrity (Graves, 2012; Kalghatgi et al., 2011).

Importantly, cells are equipped with sophisticated DNA repair mechanisms to counteract such genotoxic threats. These include base excision repair (BER), nucleotide excision repair (NER), homologous recombination (HR), and non-homologous end joining (NHEJ) (Jackson & Bartek, 2009). The repair mechanism activated depends on the type of lesion inflicted. For example, BER is efficient at resolving base damage and SSBs, while DSBs are repaired via HR or NHEJ, pathways crucial for genomic stability and cell survival (Wood, 2010).

Given the dual nature of CAP, therapeutic efficacy versus genotoxic potential understanding the molecular mechanisms by which plasma interacts with DNA and how cells respond through repair systems is of paramount importance. This knowledge is not only vital for mitigating potential risks but also for optimizing plasma-based biomedical applications so that they are safe, targeted, and effective.

Types of CAP-Induced DNA Lesions and Repair Pathways

Cold atmospheric plasma generates a milieu of charged particles and reactive molecules that can damage DNA both directly and indirectly. Direct interactions occur when plasma-generated electrons or ions physically interact with DNA strands. Indirect interactions are more common and involve oxidative stress from ROS and RNS that alter DNA bases or break phosphodiester bonds.

The table below summarizes the types of DNA damage CAP can induce, the reactive species involved, and the cellular repair pathways responsible for resolving them.

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Type of DNA Damage	Reactive Species Involved	Cellular Repair Pathways
Single-strand breaks (SSBs)	ROS, RNS	Base Excision Repair (BER)
Double-strand breaks (DSBs)	ROS	Homologous Recombination (HR), Non-Homologous End Joining (NHEJ)
Base modifications	ROS, RNS	BER, Nucleotide Excision Repair (NER)
DNA crosslinks	ROS, UV	NER
Abasic sites	ROS	BER

Table 1: CAP-induced DNA lesions and their associated repair responses.

Reactive Species and Their Impact on DNA

The figure below depicts the **relative interaction potential** of key reactive species generated by CAP with cellular DNA. These values are illustrative and based on existing empirical and computational models that assess oxidative reactivity with nucleobases and DNA backbones.

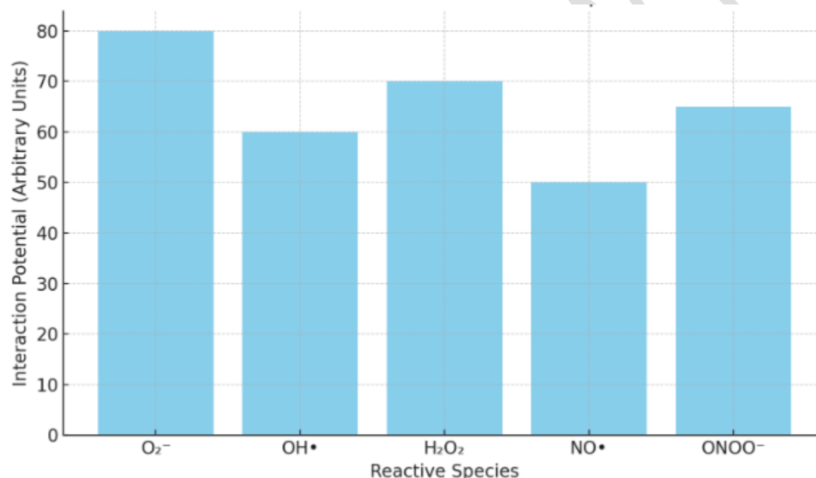

Fig. 1: Interaction potential of selected CAP-generated reactive species with DNA.

ROS such as **•OH** and **H_2O_2** are known to cause hydroxylation and strand cleavage. RNS like **NO•** and **$ONOO^-$** can nitrate DNA bases, leading to mutagenic lesions. The damage potential depends not only on the species but also on plasma exposure time, frequency, and biological context (Lu et al., 2016; Sakiyama et al., 2012).

DNA Repair Systems: The Cellular Defense

Cells employ a diverse array of DNA repair mechanisms to maintain genomic stability in the face of damage induced by agents like cold atmospheric plasma (CAP). Among these, base excision repair (BER) plays a critical role in correcting oxidized or chemically modified bases and abasic sites. This process begins with DNA glycosylases recognizing and excising the damaged bases, followed by endonuclease-mediated strand cleavage and subsequent ligation (Wood, 2010). Nucleotide excision repair (NER), on the other hand, is essential for removing bulky lesions such as thymine dimers and DNA crosslinks, making it particularly important for addressing UV- and CAP-induced distortions (Schneider et al., 2018). For more severe damage like double-strand breaks, cells rely on either homologous recombination (HR), a high-fidelity

repair pathway that uses a sister chromatid template and is active during the S and G2 phases, or non-homologous end joining (NHEJ), a faster but more error-prone process that directly ligates broken DNA ends, sometimes introducing mutations (Jackson & Bartek, 2009). A comprehensive understanding of these repair systems, including their efficiencies and limitations, is essential to assess and ensure the genomic safety of CAP, particularly when used repeatedly or in healthy tissues adjacent to treatment zones.

Relevance to Cancer Therapy and Risk Management

Interestingly, the genotoxicity of CAP is not always a liability. In fact, targeted DNA damage is beneficial in oncology, where inducing cell death in malignant cells is the therapeutic goal (Keidar, 2015). Cancer cells often have impaired DNA repair capabilities, making them more vulnerable to oxidative stress. This provides a therapeutic window to use CAP selectively. Nevertheless, ensuring minimal harm to surrounding healthy tissue requires careful dosimetry, optimized exposure conditions, and perhaps integration with DNA repair modulators to enhance selectivity (Hirst et al., 2015). This approach aligns with the concept of "plasma oncology," where CAP is being integrated with existing chemotherapeutic strategies.

The interaction of cold atmospheric plasma (CAP) with DNA offers both transformative opportunities and significant scientific challenges. While its biomedical applications—particularly in sterilization, cancer treatment, and tissue regeneration—are advancing rapidly, a deep understanding of the molecular mechanisms underlying DNA damage and cellular repair is crucial to ensure its safe and targeted use. To this end, future research must prioritize high-resolution imaging and sequencing of CAP-induced DNA lesions to precisely characterize the nature and extent of damage. Additionally, real-time monitoring of DNA repair kinetics will provide insights into cellular responses and potential repair deficiencies. Long-term studies are also necessary to evaluate the risks of mutagenesis and carcinogenicity, particularly with repeated or high-dose exposures. Furthermore, integrating CAP with DNA repair inhibitors could enhance its therapeutic efficacy, especially in oncology, by selectively increasing cancer cell vulnerability. Ultimately, the fusion of plasma physics, molecular biology, and clinical medicine is essential to unlock the full potential of CAP in biomedical science.

2. Types of Plasma-Induced DNA Damage

The interaction between cold atmospheric plasma (CAP) and cellular DNA can lead to a spectrum of molecular alterations. These damages arise primarily due to the action of plasma-generated reactive oxygen species (ROS), reactive nitrogen species (RNS), UV photons, and energetic electrons. The biological consequences depend on the nature and extent of the lesions and the efficacy of cellular repair systems. Below is a detailed exploration of the major types of DNA damage induced by CAP.

2.1 Single-Strand Breaks (SSBs)

One of the most frequently observed outcomes of plasma-DNA interaction is the formation of single-strand breaks (SSBs). These occur when the sugar-phosphate backbone of DNA is cleaved on one strand, often by hydroxyl radicals (•OH), hydrogen peroxide (H_2O_2), and other ROS present in plasma (Lu et al., 2016). SSBs are relatively less harmful than double-strand breaks; however, if left unrepaired or improperly repaired, they can interfere with transcription and replication or convert into more severe lesions like DSBs during replication stress (Sakiyama et al., 2012).

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Plasma-generated ROS attack the deoxyribose sugar or phosphodiester bonds, causing cleavage and formation of abasic sites. The repair of SSBs is primarily governed by base excision repair (BER), a pathway involving DNA glycosylases and endonucleases (Wood, 2010). While cells are generally proficient at repairing such damage, excessive exposure to plasma or impaired BER capacity can elevate the risk of mutagenesis.

2.2 Double-Strand Breaks (DSBs)

Double-strand breaks (DSBs) are among the most dangerous DNA lesions. They occur when both strands of the DNA helix are broken, either simultaneously or in close proximity. In CAP-treated cells, DSBs often result from clustered oxidative damage or direct interaction with high-energy electrons and UV photons (Fridman et al., 2008). These breaks pose a substantial threat to genomic stability, potentially leading to chromosomal rearrangements, translocations, or cell death if misrepaired.

Cells respond to DSBs via two major repair pathways: homologous recombination (HR), which is error-free but restricted to the S/G2 phases, and non-homologous end joining (NHEJ), which is faster but more error-prone (Jackson & Bartek, 2009). CAP-induced DSBs are particularly relevant in cancer therapy, where selective induction of lethal DNA breaks in malignant cells is desirable (Keidar, 2015).

2.3 Base Modifications and Oxidative Lesions

CAP-generated ROS and RNS can chemically alter DNA bases, leading to the formation of oxidized or nitrated lesions. One of the most common and well-studied oxidative lesions is 8-oxo-guanine (8-oxoG), which mispairs with adenine and results in GC to TA transversions, common mutations associated with carcinogenesis (Clancy et al., 2020). Other modified bases include thymine glycol, cytosine hydrate, and nitrosated derivatives, which can distort the DNA helix and hinder polymerase activity (Ahn et al., 2014). These lesions are primarily repaired by BER, though bulky or helix-distorting lesions may also require nucleotide excision repair (NER) mechanisms (Schneider et al., 2018). The prevalence of base modifications highlights the need to balance plasma exposure in therapeutic applications to avoid off-target genetic alterations.

2.4 Crosslinking and DNA-Protein Adducts

Plasma can also induce crosslinking within DNA (intrastrand or interstrand) or between DNA and associated proteins. These crosslinks are typically mediated by UV photons, or secondary plasma-generated electrophilic species and represent a severe form of genotoxic stress. DNA crosslinks physically block the progression of replication forks and transcription complexes, potentially triggering cell cycle arrest, or apoptosis (Laroussi, 2005).

Similarly, DNA-protein crosslinks can impair chromatin remodeling and transcription regulation. Repairing such lesions is complex and may involve a combination of NER, HR, and specialized proteases that first remove the protein adduct before repair can proceed (Benedikt et al., 2015). These damages are of particular concern in rapidly dividing cells, where replication stress can exacerbate their cytotoxicity.

Type of Damage	Primary Cause	Biological Implications
Single-Strand Breaks (SSBs)	ROS (e.g., •OH, H_2O_2)	Repaired by BER; may lead to replication stress and mutagenesis if unrepaired

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Double-Strand Breaks (DSBs)	Clustered ROS, UV, energetic electrons	High risk of chromosomal rearrangements; repaired by HR or NHEJ
Base Modifications	ROS, RNS (e.g., NO•, ONOO⁻)	Mutagenic potential; repaired by BER and sometimes NER
DNA Crosslinks	UV radiation, ROS	Blocks replication/transcription; complex repair mechanisms
DNA-Protein Adducts	Electrophilic species, peroxides	Alters gene expression; hinders repair and transcription machinery

Table 2: Summary of CAP-Induced DNA Lesions

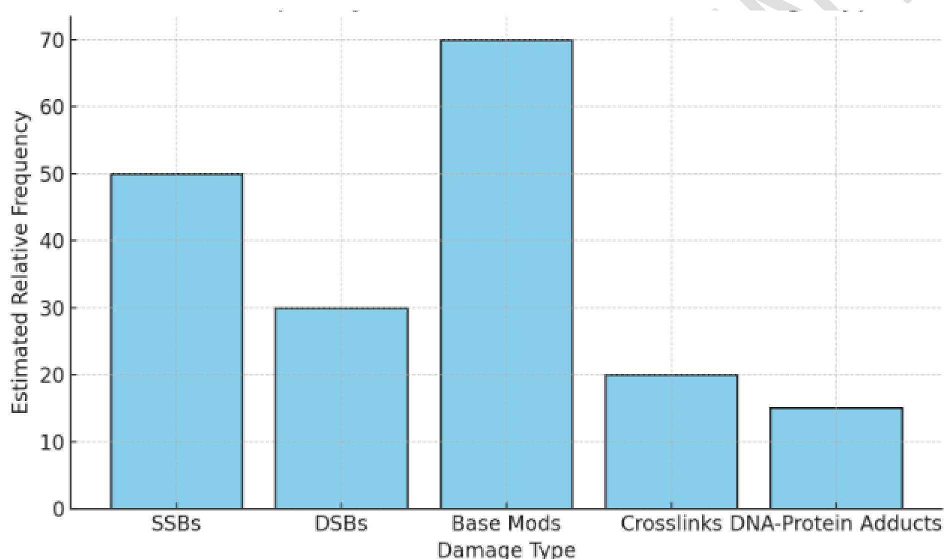

Fig. 2: Relative Frequency of Plasma-Induced DNA Damage Types

This figure emphasizes that base modifications and single-strand breaks are the most frequently observed CAP-induced lesions, followed by double-strand breaks. DNA-protein adducts and crosslinks, while less common, are often more biologically disruptive.

Cold atmospheric plasma introduces a diverse array of DNA lesions through a complex interplay of physical and chemical interactions. These range from common single-strand breaks and base modifications to severe double-strand breaks and crosslinks. The biological consequences of such damage depend on the extent of the lesion, the plasma dosage, and the capacity of cellular repair systems. While these effects pose safety challenges, they also offer therapeutic opportunities, particularly in targeting cancer cells with defective DNA repair mechanisms. A detailed understanding of the molecular basis of CAP-induced DNA damage will be essential to tailor its biomedical applications safely and effectively.

3. Reactive Species Responsible for DNA Damage

Cold Atmospheric Plasma (CAP) has emerged as a promising tool in biomedical applications, particularly in cancer therapy, wound healing, and sterilization. One of its profound biological effects involves the induction of DNA damage, a critical event that can either promote cell death in cancer cells or, conversely, pose genotoxic risks. This DNA damage is predominantly mediated by a suite of reactive species generated during plasma operation, including Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS), ultraviolet (UV) photons, and charged particles such as electrons and ions. Their combined and often synergistic effects underpin the genotoxic potential of plasma.

3.1 Reactive Oxygen Species (ROS)

Reactive Oxygen Species are chemically reactive molecules containing oxygen. Among these, hydroxyl radicals (•OH), superoxide anion radicals ($O_2^{\bullet-}$), and hydrogen peroxide (H_2O_2) are the most frequently implicated in DNA damage mechanisms following plasma exposure. Hydroxyl radicals are particularly damaging due to their extremely high reactivity and short half-life. They are known to cause base modifications, single-strand breaks (SSBs), and double-strand breaks (DSBs) by abstracting hydrogen atoms from the sugar-phosphate backbone of DNA (Fridovich, 1995).

Superoxide radicals are less reactive but can dismutate to form H_2O_2, which can further interact with transition metals via Fenton reactions to yield hydroxyl radicals (Halliwell & Gutteridge, 2015). H_2O_2 itself is relatively stable and can diffuse into the nucleus, acting as a precursor to more reactive species.

Numerous studies have highlighted that CAP-generated ROS directly induce oxidative stress in cells, evidenced by increased levels of 8-oxo-deoxyguanosine (8-oxo-dG), a biomarker of oxidative DNA damage (Wende et al., 2014). The DNA damage inflicted by ROS is often reparable; however, when overwhelming, it can trigger apoptotic or necrotic pathways.

3.2 Reactive Nitrogen Species (RNS)

Alongside ROS, plasma generates Reactive Nitrogen Species such as nitric oxide (NO•) and peroxynitrite ($ONOO^-$). RNS contribute to both nitrative and oxidative stress. Nitric oxide, although less directly genotoxic, plays a modulatory role by reacting with superoxide to form peroxynitrite, a potent nitrating and oxidizing agent capable of causing extensive DNA damage (Beckman & Koppenol, 1996).

Peroxynitrite can nitrate tyrosine residues in proteins, modify guanine bases in DNA, and induce strand breaks. DNA exposed to $ONOO^-$ shows formation of 8-nitroguanine and other mutagenic lesions. These RNS can also interact with DNA repair pathways, suppressing their functionality and amplifying damage persistence (Pacher et al., 2007).

3.3 UV Radiation and Charged Particles

The plasma environment also includes UV photons in the UVA, UVB, and UVC spectra, depending on the plasma source. These photons can directly excite DNA bases, resulting in dimer formation, especially cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts, well-known to block transcription and replication (Douki & Cadet, 2001).

Further, charged particles such as electrons and ions are inherently present in plasma. Electrons can ionize molecules or induce electronic excitation in DNA and water molecules. This leads to the generation of radicals such as hydroxyl and hydrogen radicals (Moisan et al., 2001). Ions, being massive compared to electrons, can directly strike DNA, causing significant localized

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damage including DSBs. Moreover, collisions between ions and water can produce secondary electrons which further contribute to DNA modification.

3.4 Synergistic Effects

While each component, ROS, RNS, UV, and charged particles individually can cause DNA damage, their simultaneous presence in CAP creates a highly reactive and complex environment. Studies have shown that the combined effect of ROS and RNS, often referred to as "oxidative/nitrosative stress," has a greater potential for genotoxicity than either stressor alone (Kehrer, 2000).

Synergistic interactions have been observed where UV-induced CPDs occur alongside oxidative lesions, compounding repair difficulties and increasing the risk of mutation (Sies & Jones, 2020). Additionally, electric fields generated during plasma operation can enhance the penetration and orientation of reactive species towards cellular targets, thereby intensifying the biological impact.

The collective contribution of these agents results in a spectrum of DNA damage types: base oxidation, abasic sites, single- and double-strand breaks, and crosslinking. Table 1 below summarizes these reactive species and their primary DNA interactions.

Reactive Species	Primary Effects on DNA
Hydroxyl Radical (.OH)	Base modifications, strand breaks
Superoxide (O2.-)	Precursor to other ROS
Hydrogen Peroxide (H2O2)	Forms .OH va Fenton reaction
Nitric Oxide (NO.)	Nitrostative stress, base deamination
Peroxynitrite (ONOO-)	Nitration of DNA bases, strand breaks
UV Radiation	Pyrimidine dimmers, strand breaks
Electrons	Excitation/ionization of molecules
Ions	Direct DNA collision damage
Electric Fields	Membrane potential disruption, ROS generation

Table 3 : Reactive Species and Their Effects on DNA

The full spectrum of DNA damage induced by plasma underscores the importance of understanding the interplay between various reactive species. The ability of CAP to generate targeted genotoxic effects offers great promise in selectively eliminating cancer cells while preserving healthy tissue, provided that dosage and exposure are meticulously controlled. However, further studies are necessary to delineate long-term effects, understand repair mechanisms under plasma exposure, and refine treatment parameters for clinical applications.

4. DNA Damage Detection and Quantification Techniques

The accurate detection and quantification of DNA damage are critical for evaluating the genotoxic potential of various agents, including cold atmospheric plasma (CAP). Multiple analytical and imaging-based techniques have been developed over the past decades to identify different types of DNA lesions, ranging from single-strand breaks (SSBs) and double-strand breaks (DSBs) to base modifications and DNA adducts. This section presents a comprehensive overview of the principal methods employed in DNA damage assessment, each with distinct strengths, limitations, and detection principles.

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4.1 Comet Assay

The Comet Assay, or Single-Cell Gel Electrophoresis (SCGE), is a widely adopted technique for detecting DNA strand breaks at the individual cell level. It is particularly sensitive to both SSBs and DSBs and is frequently used in genotoxicity testing. Cells are embedded in agarose, lysed to remove membranes, and subjected to electrophoresis. DNA fragments migrate toward the anode, forming a comet-like tail whose length and intensity correlate with the extent of DNA damage (Olive & Banáth, 2006).

The assay can be conducted under neutral or alkaline conditions to preferentially detect DSBs or both SSBs and DSBs, respectively. Additionally, the incorporation of lesion-specific enzymes such as formamidopyrimidine DNA glycosylase (FPG) allows for the detection of oxidative base damage (Collins, 2004). The simplicity, cost-effectiveness, and high-throughput capability of the comet assay make it an invaluable tool in plasma biology and radiation studies.

4.2 γ-H2AX Foci Formation

One of the earliest cellular responses to DSBs is the phosphorylation of the histone variant H2AX at serine 139, yielding γ-H2AX. This phosphorylation occurs in chromatin regions flanking the break sites and serves as a marker for DSBs (Rogakou et al., 1998). The resulting γ-H2AX foci can be visualized using immunofluorescence microscopy or quantified via flow cytometry.

The number and intensity of γ-H2AX foci directly correlate with the number of DSBs, making this method highly specific and sensitive. In the context of CAP treatment, γ-H2AX analysis has been employed to confirm plasma-induced genotoxicity and to evaluate cellular repair kinetics (Bonner et al., 2008). This assay is particularly useful for assessing DNA damage in tissues and fixed cells, allowing for spatial resolution within the nuclear architecture.

4.3 8-oxo-dG Assay

Oxidative stress frequently results in the formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG), one of the most prevalent and mutagenic lesions caused by ROS. The quantification of 8-oxo-dG is a gold standard for evaluating oxidative DNA damage. Multiple platforms, including enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography with electrochemical detection (HPLC-ECD), and immunohistochemistry, are employed for this purpose (Valavanidis et al., 2009).

8-oxo-dG detection is highly relevant for CAP studies, where ROS generation is a key mechanism of action. Despite the potential for background interference in biological samples, the use of proper controls and high-specificity antibodies has improved assay reliability.

4.4 LC-MS/MS and Immunoassays

Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS) is considered the gold standard for precise quantification of DNA lesions. This technique allows for the simultaneous detection of multiple base modifications and DNA adducts with high sensitivity and specificity (Cadet et al., 2010). LC-MS/MS can distinguish between isomeric lesions such as 8-oxo-dG and 8-oxo-dA and is thus valuable for comprehensive oxidative damage profiling.

Immunoassays, such as competitive ELISA and Western blotting, use antibodies to detect specific DNA damage markers like thymine dimers or alkylated bases. These techniques are less

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labor-intensive than LC-MS/MS and can be used for large-scale screening, though they typically lack the same level of chemical specificity.

Technique	Target DNA Damage	Detection Principle
Comet Assay	Single- and Double-Strand Breaks	Electrophoretic migration pattern
γ-H2AX Foci Formation	Double-Strand Breaks (DSBs)	Fluorescence-tagged phosphorylated H2AX
8-oxo-dG Assay	Oxidized Guanine Lesions (8-oxo-dG)	ELISA or HPLC-based detection of 8-oxo-dG
LC-MS/MS	Modified Bases, DNA Adducts	Mass-to-charge ratio in mass spectrometry
Immunoassays	DNA Lesions, Oxidized Nucleotides	Antibody recognition of specific DNA modifications

Table 4: Overview of DNA Damage Detection Techniques

Each technique offers unique advantages and should be selected based on the nature of the DNA damage, the biological system under investigation, and the intended resolution or sensitivity. In plasma medicine, combining multiple assays, e.g., comet assay with γ-H2AX and 8-oxo-dG detection can provide a holistic view of cellular responses and repair dynamics.

As CAP continues to be explored for clinical applications, especially in oncology, precise damage profiling becomes indispensable for safety assessment and therapeutic optimization. Future improvements in multiplexing capabilities and integration with microfluidic platforms may further advance real-time, in situ DNA damage analysis.

5. Cellular DNA Repair Pathways

Living cells are persistently challenged by endogenous and exogenous agents that cause DNA damage, ranging from oxidative stress and UV radiation to ionizing radiation and chemical mutagens. To maintain genomic integrity and prevent mutagenesis or apoptosis, cells have evolved a complex network of DNA repair mechanisms and damage response pathways. These systems detect lesions, signal their presence, and orchestrate appropriate repair. Among the primary repair mechanisms are base excision repair (BER), nucleotide excision repair (NER), non-homologous end joining (NHEJ), homologous recombination (HR), and the broader DNA damage response (DDR) network that coordinates cellular outcomes.

5.1 Base Excision Repair (BER)

BER is the predominant pathway for repairing small, non-bulky base lesions, such as those induced by reactive oxygen species (ROS), alkylation, and spontaneous deamination. It specifically targets single-base modifications and single-strand breaks (SSBs), maintaining DNA stability in response to oxidative stress, including that induced by cold atmospheric plasma (CAP).

The process is initiated by DNA glycosylases, which recognize and remove the damaged base, generating an apurinic/apyrimidinic (AP) site. AP endonuclease 1 (APE1) then cleaves the DNA backbone, followed by gap filling by DNA polymerase β and ligation by DNA ligase III, often with XRCC1 as a scaffold protein (Krokan & Bjørås, 2013). BER is rapid, accurate, and essential in both dividing and non-dividing cells. Its impairment is linked with cancer, aging, and neurodegeneration.

5.2 Nucleotide Excision Repair (NER)

NER is responsible for removing bulky, helix-distorting lesions such as UV-induced cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts. It is a versatile system that

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detects distortions in the DNA helix rather than specific base lesions. NER operates via two sub-pathways: global genomic NER (GG-NER), which scans the entire genome, and transcription-coupled NER (TC-NER), which acts on lesions that block transcription elongation (Sancar, 1996).

Key proteins involved include XPA, RPA, and TFIIH, which open the DNA around the lesion. Endonucleases XPG and XPF-ERCC1 then excise the damaged strand segment, followed by gap filling and ligation. NER is particularly important in protecting skin cells from UV-induced mutations and plays a role in CAP-treated cells when UV components are involved.

5.3 Non-Homologous End Joining (NHEJ)

Double-strand breaks (DSBs) represent one of the most lethal forms of DNA damage. NHEJ is a major pathway for DSB repair, especially in G0 and G1 phases of the cell cycle when a homologous template is not available. It is a relatively fast but error-prone process, as it can lead to insertions, deletions, or chromosomal translocations (Lieber, 2010).

NHEJ begins with recognition of DSBs by the Ku70/Ku80 heterodimer, which recruits DNA-PKcs, forming the DNA-PK holoenzyme. This complex processes DNA ends and brings them into alignment. Finally, ligation is performed by DNA ligase IV with XRCC4 and XLF. CAP-induced DSBs, confirmed by γ-H2AX foci formation, may predominantly be repaired via NHEJ in somatic cells.

5.4 Homologous Recombination (HR)

HR provides an error-free repair mechanism for DSBs by using the sister chromatid as a template. It is active primarily during the S and G2 phases of the cell cycle. HR is critical for the high-fidelity repair of breaks, maintenance of telomeres, and resolution of stalled replication forks.

HR initiates with DSB recognition by the MRN complex (MRE11-RAD50-NBS1), which recruits and activates the ATM kinase. DNA end resection follows, producing single-stranded DNA that is coated by RPA and later replaced by RAD51 to form nucleoprotein filaments. These filaments search for homologous sequences on the sister chromatid and mediate strand invasion and repair synthesis (Jasin & Rothstein, 2013). The HR pathway is vital in stem cells and rapidly dividing tissues and is also modulated in cancer therapies and CAP interventions.

5.5 DNA Damage Response (DDR)

DDR is an overarching surveillance system that senses DNA damage and activates downstream pathways to halt the cell cycle, repair lesions, or trigger apoptosis if repair fails. Central to DDR are three phosphoinositide 3-kinase-related kinases (PIKKs): ATM, ATR, and DNA-PKcs.

ATM responds primarily to DSBs and activates CHK2 and p53, leading to G1 arrest or apoptosis. ATR, activated by replication stress and single-stranded DNA, activates CHK1 to mediate S/G2 arrest. DNA-PKcs is more closely associated with NHEJ. These kinases orchestrate a complex signaling cascade involving chromatin remodeling, repair protein recruitment, and transcriptional reprogramming (Ciccia & Elledge, 2010). In CAP-treated cells, the DDR determines the fate of cells repair, senescence, or death, depending on the extent of DNA damage and repair capacity.

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Repair Pathway	Primary Function	Key Enzymes/Proteins
Base Excision Repair (BER)	Repairs small, non-helix-distorting base lesions	DNA glycosylases, APE1, DNA polymerase β, XRCC1
Nucleotide Excision Repair (NER)	Removes bulky, helix-distorting lesions like thymine dim	XPA, RPA, TFIIH, XPF-ERCC1, XPG
Non-Homologous End Joining (NHEJ)	Repairs double-strand breaks without a template; fast b	Ku70/80, DNA-PKcs, Ligase IV, XRCC4
Homologous Recombination (HR)	Uses homologous template to repair DSBs accurately	RAD51, BRCA1/2, MRN complex, ATM
DNA Damage Response (DDR)	Senses DNA damage and coordinates checkpoint contro	ATM, ATR, DNA-PK, CHK1/2, p53

Table 5: Overview of DNA Repair and Response Pathways

Understanding the mechanistic details of these pathways is critical not only for appreciating cellular resilience but also for exploiting repair deficiencies in cancer cells. For example, targeting PARP in BRCA-mutated cancers impairs BER and HR, leading to synthetic lethality. Similarly, modulating DDR pathways may enhance the selectivity and efficacy of CAP in tumor ablation.

Moreover, defects in any of these pathways can lead to hypersensitivity to radiation or chemicals and are implicated in numerous hereditary syndromes, including xeroderma pigmentosum (NER defect), ataxia telangiectasia (ATM defect), and Nijmegen breakage syndrome (NBS1 defect).

6. Influence of Plasma Parameters on DNA Damage

The extent and nature of DNA damage induced by cold atmospheric plasma (CAP) are tightly governed by a constellation of operational parameters. Understanding this interplay is critical for both therapeutic applications and biosafety considerations.

6.1 Plasma Type and Source

Dielectric barrier discharges (DBDs) and plasma jets generate RONS via distinct physical mechanisms, influencing both the species composition and tissue penetration. DBDs created between electrodes separated by a dielectric barrier tend to produce a rich mixture of short-lived species (•OH, O_3, ^1O_2) and deeper UV components, but exhibit limited penetration (~sub-mm) into tissues. In contrast, jet devices, often driven by helium or argon, propel long-lived RONS deep into liquid media or tissue due to their momentum and admixture with ambient air. Consequently, DNA strand breaks and base modifications such as 8-oxoG are more pronounced in jet-treated samples, as these species induce oxidative DNA damage both directly via radicals and indirectly via H_2O_2.

6.2 Treatment Time and Distance

Exposure duration and proximity strongly modulate DNA damage levels. Longer durations and shorter gap distances elevate local RONS density, increasing both single and double-strand DNA breaks. For instance, in He-jet-treated HCT116 spheroids, 240 s treatment induced over 2 mM H_2O_2 in conditioned media, correlating with pronounced γ-H2AX foci and DNA fragmentation. DNA damage was largely reversible by catalase, highlighting H_2O_2's central role. Additionally, in cell monolayers treated with nitrogen-based APP, γ-H2AX staining decreased with increasing distance from the nozzle, while comet assays showed quantitative fragmentation at 0.5 cm but full breaks at 0.1 cm in just seconds.

6.3 Carrier Gas Composition

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Helium, argon, oxygen admixtures, or humidified gases influence both the type and abundance of reactive species. He plasmas produce high densities of metastables and energetic electrons that readily generate •OH and singlet oxygen via energy transfer to ambient air, EPR studies confirm elevated •OH in He-treated DMEM compared to argon, or air plasmas. Argon, while supporting similar ROS generation, can modulate kinetics and yields differently; surfacingly, humidified argon jets produced greater DNA damage and antimicrobial effects than dry variants. Oxygen admixtures further influence nitration chemistry, generating RNS species like NO_2^-, peroxynitrite, which also contribute to DNA oxidation.

6.4 Environmental Conditions

Ambient humidity, temperature, and presence of biomolecules significantly alter plasma reactivity and resultant DNA damage. Humidified He or Ar feeds support enhanced •OH production via water vapor reactions; e.g., humid argon increased microbial killing and DNA damage compared to dry gas. Temperature influences both radical lifetimes and cell sensitivity; higher temperatures can degrade RONS or increase cell susceptibility. Additionally, biomolecules such as amino acids and proteins in the medium act as RONS sinks or secondary radical sources. Plasma-activated media (PAM) studies show that amino acids in culture fluids can themselves generate long-lived radicals, amplifying DNA damage in subsequent cellular exposures . For example, PAM stored at +4 °C retained DNA-damaging ability for days, whereas higher storage temperatures diminished its potency.

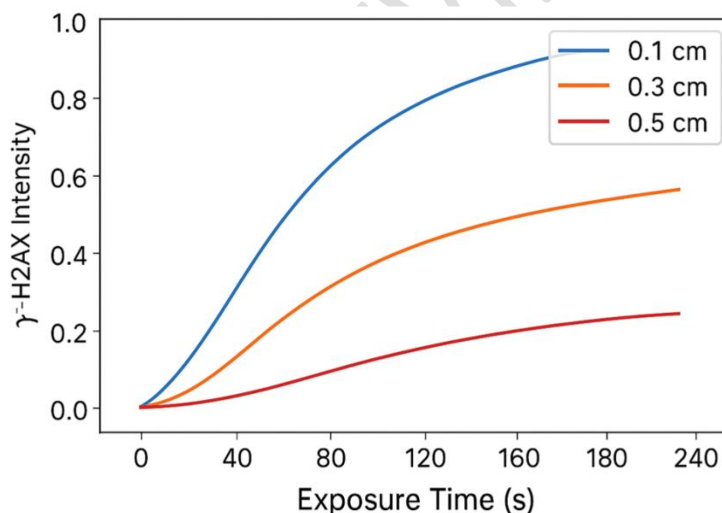

Fig. 3 : Dose–Response Curve: γ-H2AX intensity vs. exposure time at various distances.

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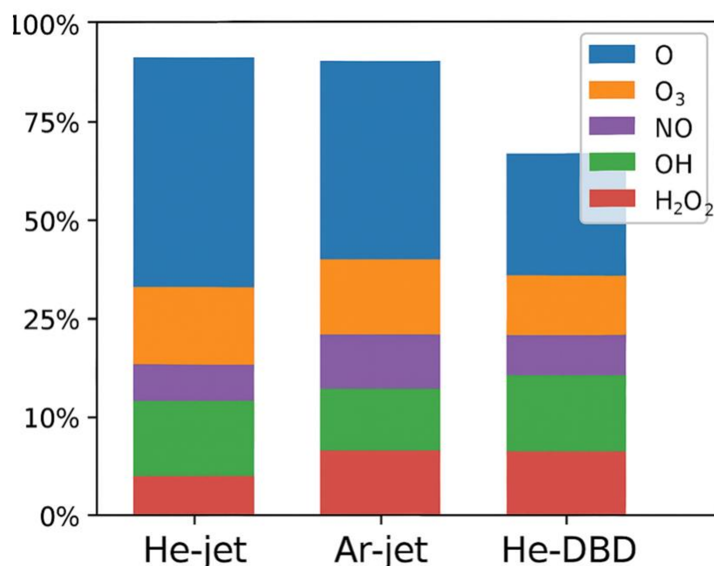

489 **Fig. 4 :** Comparison of RONS generated by He-jet, Ar-jet, and He-DBD.

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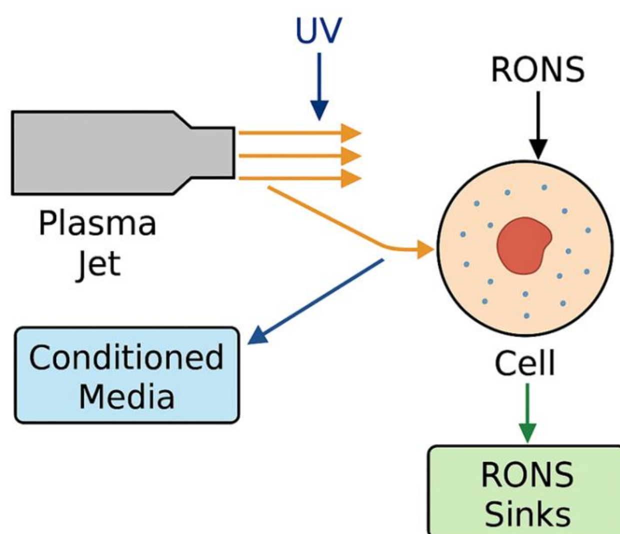

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493 **Fig. 5 :** Interaction pathways, direct plasma, conditioned media, UV, and RONS sinks.

494

495 CAP-induced DNA damage is highly tunable via careful modulation of plasma type,
496 exposure time, device-to-sample distance, gas composition, and environmental context. He-jet
497 plumes produce deeper and broader DNA damage via abundant •OH and H_2O_2; humidified argon
498 can elicit even stronger genotoxic effects. Media composition prolongs the lifetime and diffusion
499 of RONS in conditioned fluids. These insights allow fine control over plasma's biological
500 impact, offering a mechanism to either harness DNA damage in cancer therapy or minimize it in
501 tissue-regenerative applications. Continued quantitative characterization across these parameters
502 is crucial for ensuring safe, effective CAP deployment in clinical and industrial settings.

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7. Biological Context and Implications

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Cold Atmospheric Plasma (CAP) represents an innovative approach in biomedical applications, particularly in oncology, due to its selective cytotoxicity towards cancer cells. However, to fully realize its therapeutic potential, it is essential to address the biological mechanisms underpinning this selectivity, evaluate its effects on healthy cells, explore emerging applications such as gene editing, and conduct long-term safety studies.

7.1 Selectivity Toward Cancer Cells

Cancer cells exhibit a heightened sensitivity to CAP due to several intrinsic vulnerabilities. These include elevated basal levels of reactive oxygen and nitrogen species (RONS), impaired antioxidant defense systems, and deficient DNA repair mechanisms. Kim and Chung (2016) demonstrated that helium-fed CAP jets caused significant apoptosis in A549 lung carcinoma cells but had negligible effects on normal cells. The study attributed this selectivity to increased intracellular NO and NO_2^- levels, combined with lower catalase activity in cancer cells. Similarly, Sun et al. (2014) showed that CAP treatment selectively disrupted head and neck squamous carcinoma cell lines while sparing normal oral epithelial cells.

Yan et al. (2017) expanded this understanding by highlighting the role of aquaporin channels in cancer cells, which facilitate the uptake of plasma-generated H_2O_2, leading to mitochondrial dysfunction and apoptotic cascades. In a preclinical in vivo model of cholangiocarcinoma, Vaquero et al. (2020) reported that CAP treatment activated DNA damage response pathways, such as p53 and CHK1, culminating in tumor regression without affecting surrounding healthy tissues. These findings suggest that the redox imbalance and compromised repair mechanisms of cancer cells render them more vulnerable to plasma-induced oxidative stress (Graves, 2012).

7.2 Risk to Healthy Cells

While CAP exhibits selectivity, its application must be carefully controlled to avoid unintended damage to healthy tissues. Brehmer et al. (2021) evaluated the long-term impact of monthly CAP exposure on mouse oral mucosa and found no signs of inflammation or preneoplastic lesions, supporting its safety under controlled conditions. However, Zhang et al. (2021) observed that excessive CAP exposure in murine fibroblast cultures led to DNA damage and decreased proliferation, although the treatment did not induce mutations in Ames tests. This duality underscores the need for precise parameter optimization. Factors such as treatment duration, plasma jet distance, carrier gas composition, and tissue type influence the degree of RONS interaction with cells. For instance, Bekeschus et al. (2016) used the HET-MN model and showed no genotoxic effects with argon-based plasma jets, suggesting that not all plasma configurations pose equal risks.

7.3 Potential in Gene Editing

A novel yet largely unexplored application of CAP is in the realm of gene editing. CAP generates specific DNA lesions such as single- and double-strand breaks, 8-oxo-guanine, and other oxidative modifications, potentially useful for stimulating targeted repair mechanisms. Though still theoretical, coupling CAP-induced damage with base editors or homologous templates could lead to innovative gene editing methods. However, no direct studies have yet validated CAP’s utility for precision genome modification, and significant research is needed to understand its molecular specificity and off-target risks (Graves, 2012).

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7.4 Long-Term Genotoxicity Studies

One of the most critical safety concerns in CAP application is its long-term genotoxic potential. While short-term studies have reported favorable outcomes, chronic exposure needs more extensive investigation. In a one-year murine model, Brehmer et al. (2021) found that repeated CAP exposure to oral mucosa did not induce histological or genetic abnormalities. Similarly, a five-year follow-up by Metelmann et al. (2020) on patients treated with CAP for skin lesions reported no adverse effects, such as inflammation or tissue dysplasia.

However, these findings, while encouraging, are limited by small sample sizes and device variability. Yan et al. (2017) emphasized the need for standardized, long-term assays including whole-genome sequencing to monitor subtle mutations, genomic instability, and potential carcinogenesis. Only with such comprehensive safety evaluations can CAP be safely integrated into mainstream therapeutic protocols.

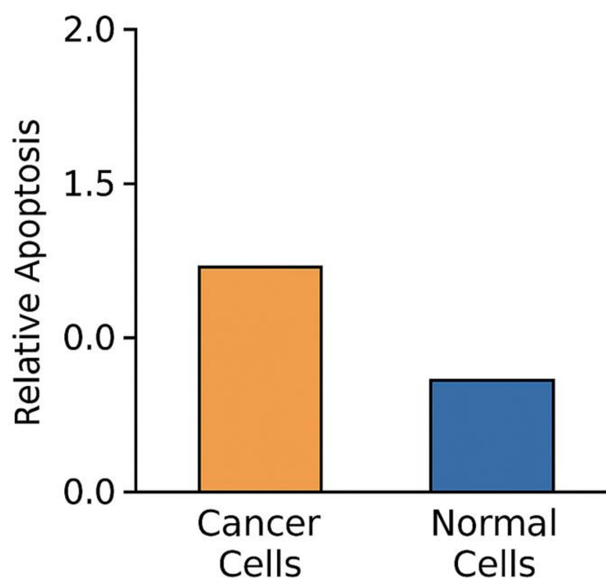

Fig. 6 : Bar chart of relative apoptosis in cancer vs. normal cells post-CAP.

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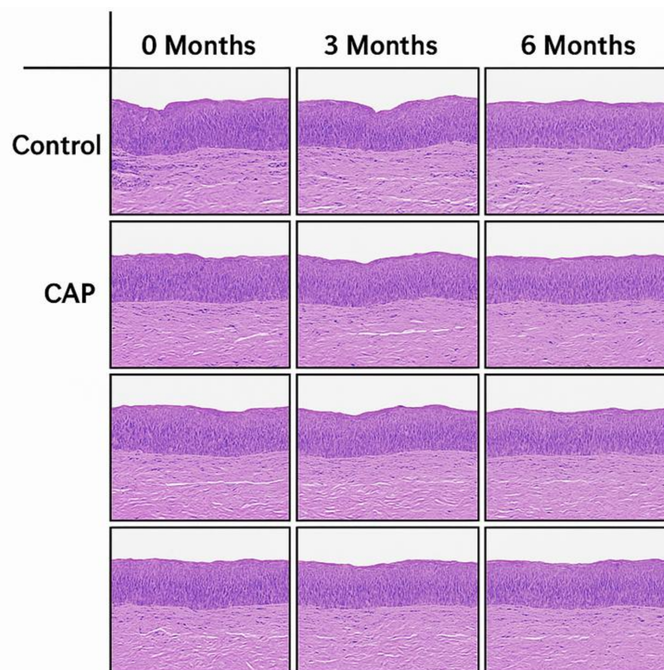

569 **Fig. 7** : Longitudinal tracking of mucosal histology in mice exposed to monthly CAP vs.
570 controls.
571

572 ## 8. Current Challenges and Future Directions

573 Despite the promising advances in plasma medicine, several critical challenges must be
574 addressed before cold atmospheric plasma (CAP) therapies can become standard clinical tools.
575 One of the foremost issues is balancing efficacy and safety. The therapeutic success of CAP
576 hinges on the generation of reactive oxygen and nitrogen species (RONS), which induce
577 oxidative stress in targeted cells, especially cancer cells. However, an overabundance of RONS
578 or prolonged exposure can also damage healthy tissues, causing unwanted genotoxicity. The
579 intricate interplay between dose, exposure time, distance from the target, and plasma
580 composition must be meticulously optimized. For example, shorter treatment times and larger
581 distances often reduce cytotoxic effects but may compromise antitumor efficacy. The
582 development of patient-specific treatment planning models and adaptive plasma devices that can
583 modulate intensity in real time could be pivotal in achieving this balance. Additionally,
584 incorporating selective targeting strategies, such as nanoparticle-enhanced delivery or molecular
585 shielding of normal tissues, might enable safer application of plasma in sensitive clinical settings
586 such as oral, dermal, and mucosal tissues.

587 Another crucial frontier is the real-time monitoring of DNA damage. CAP's effects on
588 nucleic acids, particularly DNA double-strand breaks, oxidative base lesions like 8-oxoG, and
589 histone modifications occur rapidly and vary with cellular context. Current evaluation methods
590 are largely endpoint-based, such as immunofluorescence detection of γ-H2AX foci or comet
591 assays, which only provide snapshots of cumulative damage. To safely apply CAP in clinical
592 practice, researchers must develop in situ biosensors or imaging systems capable of detecting and
593 quantifying DNA damage and repair dynamics in real time. Fluorescent nanoparticle reporters,
594 live-cell reporters of DDR activation (e.g., p53-GFP constructs), and label-free techniques like
595 Raman spectroscopy and photoacoustic imaging are being explored for this purpose. These
596 technologies would allow clinicians to titrate CAP exposure precisely and intervene if excessive

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597 genotoxic thresholds are approached. Additionally, understanding how plasma-induced DNA
598 lesions are processed by different DNA repair pathways, such as homologous recombination
599 (HR), non-homologous end joining (NHEJ), and base excision repair (BER) will aid in defining
600 the limits of reversible vs. irreversible damage, thereby guiding clinical dosimetry protocols.
601 The complexity of plasma-biomolecule interactions presents yet another significant
602 challenge. While numerous studies have shown CAP's effects on nucleic acids and membrane
603 lipids, there is still a limited understanding of how plasma influences chromatin architecture,
604 histone tail modifications, and broader epigenetic regulatory systems. These components are
605 integral to gene expression regulation, and subtle alterations could have long-lasting phenotypic
606 consequences. For instance, reactive species can modify histone side chains (e.g., lysine
607 acetylation or methylation), potentially silencing or activating genes aberrantly. In stem cells or
608 immune cells, such unintended epigenetic changes might disrupt differentiation or
609 immunomodulatory functions. Moreover, chromatin compaction affects how accessible DNA is
610 to RONS and plasma-induced radicals. Thus, variations in chromatin state could influence CAP
611 efficacy across cell types and tissue microenvironments. Advanced techniques such as ATAC-
612 seq, ChIP-seq, and Hi-C, integrated with plasma treatment studies, will be crucial to unravel
613 these mechanisms. Further, exploration of the plasma-induced modulation of non-coding RNAs,
614 such as miRNAs and lncRNAs may reveal yet another layer of regulatory complexity
615 influencing CAP responses.
616 Finally, a major hurdle to widespread adoption is regulatory and clinical translation. At
617 present, CAP remains largely an experimental tool, with limited approved applications in wound
618 healing, dermatology, and dentistry. There is a pressing need for standardized operating
619 protocols, quality assurance frameworks, and comprehensive safety guidelines. Parameters such
620 as plasma device calibration, gas type, voltage, frequency, treatment time, and patient-specific
621 considerations must be universally defined and documented. Moreover, the diverse array of
622 plasma devices ranging from dielectric barrier discharges (DBD) to handheld plasma jets,
623 necessitates cross-platform comparisons and harmonized documentation. In this context,
624 international collaborative efforts, such as those coordinated by the International Society for
625 Plasma Medicine (ISPM), are essential to developing consensus standards. On the regulatory
626 front, extensive toxicological and mutagenicity data are required by agencies such as the FDA
627 and EMA before plasma therapies can be approved for oncological or systemic use. Long-term
628 animal studies, human pilot trials, and risk assessments focused on immune compatibility and
629 genomic stability must be undertaken. Also, integration with existing medical workflows—such
630 as endoscopic delivery for gastrointestinal tumors or catheter-based application for vascular
631 targets will be essential for practical implementation.
632 While the therapeutic potential of CAP is undeniable, transitioning from bench to bedside
633 requires a multifaceted effort. Optimization of plasma delivery to ensure effective yet safe
634 dosing, development of real-time biosensors for genotoxic surveillance, in-depth exploration of
635 molecular and epigenetic mechanisms, and robust regulatory frameworks are key priorities. With
636 advances in precision plasma engineering, omics technologies, and clinical integration tools,
637 these challenges can be systematically addressed, paving the way for CAP's inclusion in the
638 future therapeutic arsenal of biomedicine.
639

640
9. Conclusion

641 Cold Atmospheric Plasma (CAP) represents an emerging and highly promising
642 technology within the realm of biomedicine, particularly due to its ability to induce DNA

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damage and modulate cellular responses. The interaction between plasma-induced reactive species and genetic material lies at the heart of many of its therapeutic applications. As our understanding deepens, the significance of these interactions becomes increasingly apparent—not just in promoting cytotoxicity toward cancerous tissues but also in determining the long-term safety and viability of CAP-based clinical therapies. This dual-edged nature of DNA damage, both as a therapeutic tool and as a potential risk, underscores the importance of this area in plasma medicine.

At the molecular level, CAP generates a cocktail of reactive oxygen and nitrogen species (RONS), including hydroxyl radicals (•OH), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and nitric oxide (NO). These species can diffuse into cells and interact with nucleic acids, inducing a spectrum of lesions such as single-strand breaks, double-strand breaks, base modifications (e.g., 8-oxo-guanine), and DNA–protein crosslinks. These lesions, when overwhelming or improperly repaired, can lead to cell cycle arrest, apoptosis, or senescence effects that are beneficial in targeting cancer cells. Indeed, this mechanism has been harnessed in several in vitro and in vivo models to suppress tumor growth, demonstrating selective cytotoxicity toward malignant cells with minimal impact on adjacent normal tissues.

However, the very mechanisms that make CAP effective in cancer therapy also raise safety concerns, especially in non-cancer applications. Persistent or misregulated DNA damage responses (DDR) can result in genomic instability, a hallmark of many chronic diseases including cancer. Therefore, understanding the thresholds between therapeutic damage and genotoxicity is critical. Several studies have begun to explore this by examining biomarkers like γ-H2AX, p53 phosphorylation, and ATM/ATR pathway activation following CAP exposure. These molecular signatures help determine the extent of DNA damage and the competence of the cell's repair mechanisms. Importantly, normal cells often exhibit robust DNA repair capabilities, which may explain their relative resistance to CAP-induced cytotoxicity. Conversely, cancer cells often harboring mutations in p53, BRCA1/2, or mismatch repair genes may lack the ability to effectively repair even modest levels of DNA damage, thus rendering them more vulnerable to plasma treatments.

Despite these encouraging findings, there remain significant gaps in our knowledge. For instance, the influence of plasma parameters, such as exposure time, device type (dielectric barrier discharge vs. plasma jet), carrier gas composition (helium, argon, oxygen), and treatment distance on DNA damage profiles is still not fully understood. These factors influence the concentration and lifetime of reactive species, and subsequently, their ability to penetrate tissues and induce genetic modifications. The heterogeneity of biological systems adds another layer of complexity: tissues vary in antioxidant capacity, chromatin structure, cell cycle distribution, and microenvironmental conditions, all of which affect CAP responsiveness.

To address these challenges, interdisciplinary research combining plasma physics, molecular biology, bioengineering, and clinical sciences is needed. New tools are being developed to monitor DNA damage in real-time, such as live-cell imaging systems for DDR markers, biosensors that detect oxidative lesions, and transcriptomic profiling to assess gene expression changes post-treatment. These innovations could enable clinicians to dynamically adjust plasma dosing based on the observed biological response, thereby enhancing safety and efficacy.

Long-term studies are also essential to assess the genomic integrity of CAP-treated tissues. While short-term data suggests minimal mutagenic potential, especially in healthy cells, comprehensive longitudinal studies using animal models and clinical cohorts are necessary to

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689 rule out delayed effects such as carcinogenesis, immune dysregulation, or epigenetic remodeling.
690 Regulatory agencies will likely demand such evidence before approving CAP-based therapies for
691 routine clinical use.

692 Equally important is the integration of CAP within broader therapeutic strategies.
693 Combining CAP with chemotherapeutics, radiotherapy, or nanoparticle-based delivery systems
694 may enhance outcomes through synergistic mechanisms. For instance, plasma may sensitize
695 resistant cancer cells to chemotherapy by disrupting DNA repair pathways or altering membrane
696 permeability. Similarly, pre-treating tissue with plasma may enhance drug absorption or
697 stimulate immune cell recruitment, thus broadening the scope of its therapeutic applications
698 beyond oncology to include wound healing, dermatological conditions, and antimicrobial
699 treatments.

700 In conclusion, the study of plasma-induced DNA damage and repair mechanisms is a
701 cornerstone of advancing CAP from a laboratory innovation to a clinical reality. The future of
702 plasma medicine hinges on our ability to control and fine-tune these interactions—maximizing
703 therapeutic benefit while minimizing unintended consequences. With continued investment in
704 research, technological refinement, and clinical validation, CAP has the potential to become a
705 powerful, safe, and precise modality in modern medicine.

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