Review

Cold Atmospheric Plasma for Breast Cancer

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Maminul Islam [1,2], Xiao Chen [1,2], Mingzhu Liu [1,2], Xi Tang [1], Fei Cao [1,*], Denis B. Zolotukhin [3], Zhaowei Chen [4] and Zhitong Chen [1,2,5,6,*]

[1] Paul C. Lauterbur Research Center for Biomedical Imaging, Institute of Biomedical and Health Engineering, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

[2] University of Chinese Academy of Sciences, Shenzhen 518055, China

[3] Tomsk State University of Control Systems and Radioelectronics, 40 Lenin Ave., Tomsk 634050, Russia

[4] Institute of Food Safety and Environment Monitoring, MOE Key Laboratory for Analytical Science of Food Safety and Biology, College of Chemistry, Fuzhou University, Fuzhou 350108, China

[5] Advanced Therapeutic Center, National Innovation Center for Advanced Medical Devices, Shenzhen 518100, China

[6] State Key Laboratory of Biomedical Imaging Science and System, Shenzhen 518055, China

* Correspondence: zt.chen1@siat.ac.cn (Z.C.); f.cao@siat.ac.cn (F.C.)

Abstract

Globally, the burden of breast cancer remains high as it is the most prevalent malignancy among women and a major contributor to cancer mortality, with therapeutic success often limited by drug resistance, treatment toxicity, and tumor heterogeneity. Cold atmospheric plasma (CAP), a partially ionized gas enriched in reactive oxygen and nitrogen species (RONS) electromagnetic waves and ultraviolet radiation, has emerged as a selective antitumor therapy, inducing cancer-specific cytotoxicity while sparing normal tissue. Here, we review the mechanisms of CAP action against breast cancer, including RONS-mediated oxidative stress, mitochondrial disruption, apoptosis, immunogenic cell death, and suppression of metastatic and angiogenic pathways. Notably, This approach selectively targets therapy-resistant breast cancer stem cells and sensitizes the highly aggressive forms, particularly triple-negative breast cancer (TNBC). Its synergy with drug therapy, radiotherapy, immunotherapy and surgery further broadens therapeutic potential. Advances in delivery platforms, such as plasma-activated media, nanoparticles, and hydrogels, address CAPs instability and enhance tumor penetration. Despite promising preclinical results, clinical translation faces barriers such as the short half-life of RONS, device standardization, and unresolved immunomodulatory effects. Overcoming these challenges through interdisciplinary collaboration and optimized protocols may unlock the potential of CAP for precision oncology.

Keywords: cold atmospheric plasma (CAP); breast cancer; RONS; immunogenic cell death; combination therapy

1. Introduction

A class of diseases known as cancer is defined by the unchecked and abnormal growth of cells, which can develop into tumors or spread throughout the body [1]. Malignancies occur in many forms, with common examples including breast, lung, skin, and hematological cancers [2]. With an estimated 670,000 deaths reported in 2022, breast cancer remains the most common malignancy affecting women worldwide. It presents a major socioeconomic and public health challenge [3]. The growing incidence and mortality observed in both developed and developing countries have contributed to breast cancer becoming one of the most frequently diagnosed cancers worldwide [4]. Based on biomarkers like hormone receptors and HER2 status, breast cancer is divided into four molecular subtypes: triple-negative, HER2$^+$, luminal A, luminal B and normal-like (ER$^+$). Each subtype has unique progression patterns, treatment responses, risk factors, and metastasis sites [5].

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To overcome challenges such as metastasis and resistance to therapy, breast cancer treatment commonly requires a multidisciplinary approach involving multiple modalities, including surgery, chemotherapy, radiotherapy, endocrine therapy, immunotherapy, and targeted therapy, all of which require collaboration among multiple medical specialties [6]. While these therapies are frequently used, some limitations impede their expanded applications. For example, targeted therapies, favored by current guidelines for prolonging survival and enhancing long-term effects in metastatic cancer [7]. These therapies, may have significant short- and long-term adverse effects such as cardiovascular problems, the possibility of secondary cancers, neuropathy, anemia, bone marrow suppression, blood clotting, and psychological problems [8]. To effectively manage breast cancer, treatments should be specifically targeted to the breast, as systemic administration is not required and could introduce additional risks [9]. Derived from the Greek word "πλάσμα," plasma was first characterized in 1928 by Irving Langmuir, who recognized it as the fourth fundamental state of matter, separate from solid, liquid, and gaseous phases [10]. As a crystalline solid is heated, its molecular bonds weaken and eventually break, melting it into a liquid, which then becomes a gas with further heating, and then, with more energy, plasma is generated as energetic collisions take electrons away from atoms. After this, heating alone does not produce any other state of matter [11]. Based on Maxwell–Boltzmann thermodynamic equilibrium, plasmas can be divided into thermal and nonthermal types. Cold atmospheric plasma (CAP) represents a form of nonthermal plasma that is generated at low temperatures under atmospheric pressure conditions [12]. Atmospheric-pressure plasma jets (APPJs), Dielectric barrier discharges (DBD), and corona discharges are commonly used devices for generating CAP (Figure 1a) [13–16]. Although the precise biological processes underlying plasma-cell interactions have not been completely elucidated, CAP-induced biological effects are primarily attributed to the activity of RONS [17,18]. Plasma discharge generates a variety of reactive oxygen species (ROS), including hydroxyl radicals (•OH), hydrogen peroxide (H_2O_2), superoxide anions (O_2^-•), singlet oxygen ($^1O_2$), and ozone (O_3). In addition, CAP leads to the formation of several reactive nitrogen species (RNS), including nitric oxide (•NO), nitrogen dioxide (•NO_2), dinitrogen tetroxide (N_2O_4), nitrogen trioxide (NO_3), nitrous oxide (N_2O), and peroxynitrite ($ONOO^-$), along with physical components such as electromagnetic radiation, ultraviolet (UV) photons, electric fields, and high-energy electrons (Figure 1b) [16,19].

As CAP overcomes the limitations associated with high-temperature plasma systems, it has garnered significant attention in medical and biological appliations. The capacity of CAP technology to provide a minimally invasive option that permits selective cancer cell death without causing harm to healthy tissue is one of its main advantages. CAP has become an excellent biomedical tool for targeted cancer therapy in recent years. Through an analysis of its selective anticancer mechanisms, which are mediated by RONS, this review systematically examines the therapeutic efficacy of CAP as a treatment modality for breast cancer. These processes include killing cancer cells, immunomodulation, microenvironment remodeling, and Immunogenic cell death (ICD) etc, (Figure 1c). Furthermore, the review explores CAP's immunomodulatory capabilities, particularly its role in triggering immunogenic cell death and enhancing immune checkpoint therapies. In addition, this review outlines potential strategies to overcome drug resistance and enhance treatment efficacy, particularly through the integration of CAP with conventional therapies (Figure 1d). It also addresses key translational challenges associated with clinical implementation, including protocol standardization, delivery optimization, and safety considerations. Finally, it suggests practical research avenues to facilitate the translation of experimental evidence into healthcare practice.

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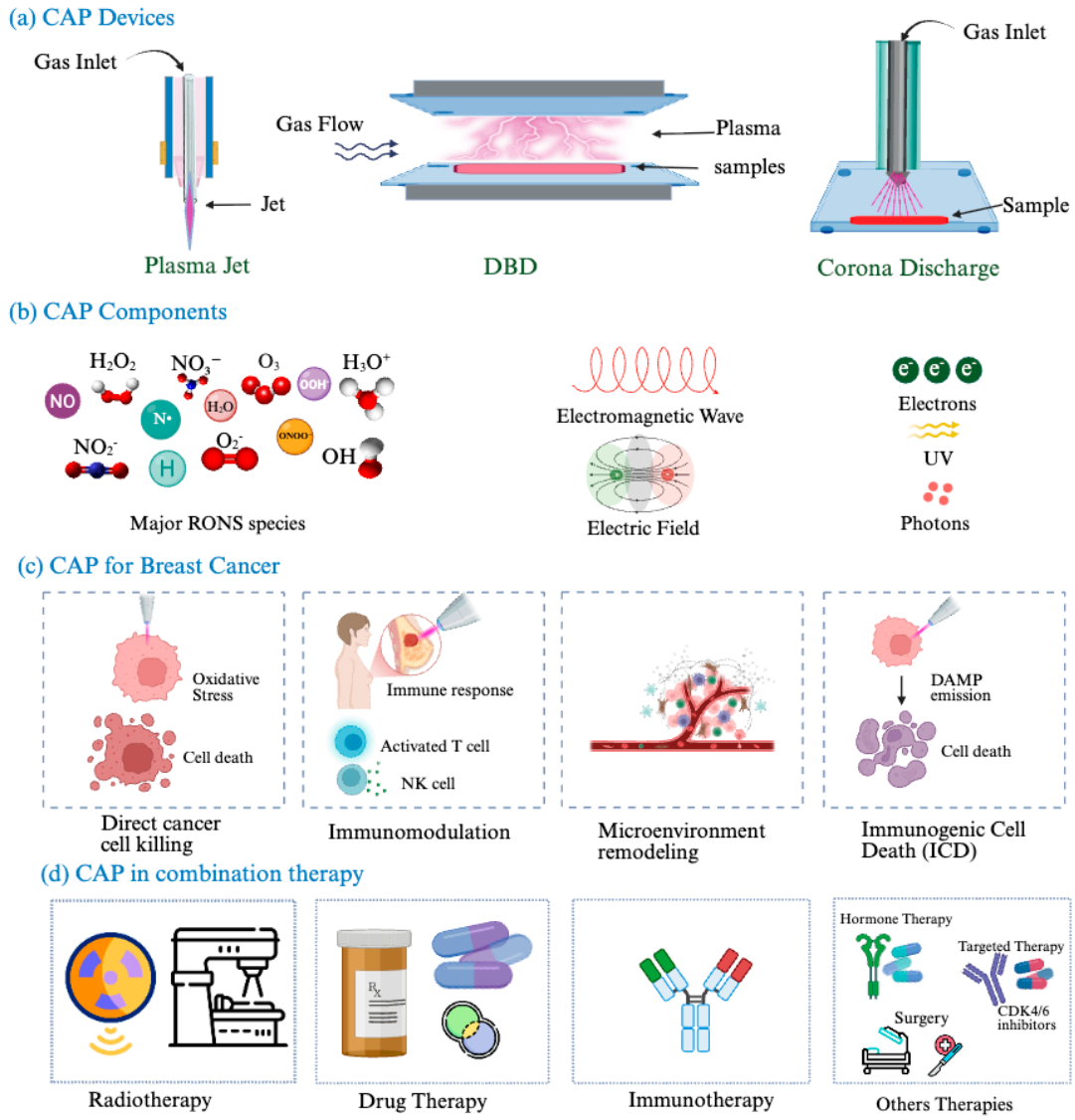

Figure 1. (a) Classification of typical CAP implementations: plasma jet, dielectric barrier discharge (DBD) and corona discharge; (b) Plasma reactive species (ROS/RNS), UV light, electromagnetic waves, and electric fields: (c) The role of CAP in breast cancer treatment: (d) Synergistic integration of CAP with conventional therapies for breast cancer treatment. Figure created by BioRender.com.

2. Cold Atmospheric Plasma (CAP): Plasma Technology and Mechanisms of Action in Cancer

Current research relies on a select group of well-established atmospheric-pressure plasma technologies that have been extensively refined for biological applications to generate CAP [13]. Short-lived radicals and longer-lived molecular oxidizing agents are among the complex mixture of RONS that are produced by plasma discharges [19]. The reactive species generated during CAP treatment are crucial mediators of its therapeutic and biological effects, although the exact ways they interact with cellular components are still being explored. CAP is considered an innovative strategy to anticancer treatment because it is able to selectively affect tumor cells while leaving healthy tissue largely unharmed, unlike conventional therapies which, often lacks such selectivity.

One conclusion suggests that cancer cells are more vulnerable because they already possess elevated intracellular ROS compared with normal cells [20]. Cancer cells contain more membrane cholesterol, and HO_2- driven oxidative reactions mainly target the double-bond region of cholesterol. Importantly, Selective tumor cell damage may occur as oxidative stress is amplified by long-lived reactive species generated during CAP treatment, such as H_2O_2 and NO_2^- [21,22]. This selective

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oxidation weakens and disrupts the cancer cell membrane, creating structural gaps that facilitate additional ROS to influx into the cell and amplify the damage [23]. Additionally, Expression levels of p53, a regulator of cell death, may influence the sensitivity of cancer cells to CAP therapy [24,25]. RONS are natural byproducts of cellular metabolism, especially during oxygen use. RONS also serve as signaling molecules at low levels, regulating processes like growth, survival, and programmed death. In excess, however, they cause oxidative stress, damaging DNA, proteins, and membranes. Maintaining appropriate levels is therefore crucial, as these molecules perform roles that influence both normal biological processes and disease development [26]. Because higher intrinsic oxidative stress levels render cancer cells more vulnerable to RONS-induced cell death than normal cells [27,28]; this imbalance causes necrosis (cell swelling, rupture) and apoptosis (cell shrinkage, DNA fragmentation) [27], ferroptosis, pyroptosis and cell-cycle arrest. RONS, including superoxide anions, are necessary for synthesizing other reactive species and can damage proteins, mitochondria, the endoplasmic reticulum, cell membranes, and the nucleus. They also have a longer half-life than other molecules and react with other molecules much more quickly. RONS harness signaling molecules to modify death pathways [27] (Figure 2). Severe cellular damage can result in necrosis or apoptosis, which are two ways that cells die [29]. Cellular stresses, including oxidative damage, radiation, and cytotoxic drugs, can activate the mitochondria-dependent intrinsic apoptotic pathway. Apoptosis is initiated when Bax and Bak activation leads to mitochondrial membrane permeabilization and the subsequent release of cytochrome c into the cytosol. The interaction between death ligands—including TNF, Fas-L, and TRAIL—and their corresponding receptors on the cell surface, such as TNFR1, Fas (CD95/Apo-1), and TRAIL receptors, initiates the extrinsic apoptosis pathway and ultimately results in cell death [25,30]. Distinct expression patterns of NOX1, catalase, and SOD in advanced malignant cells may contribute to the tumor-selective activity of CAP, as lipid peroxidation by RONS requires prior inactivation of catalase.

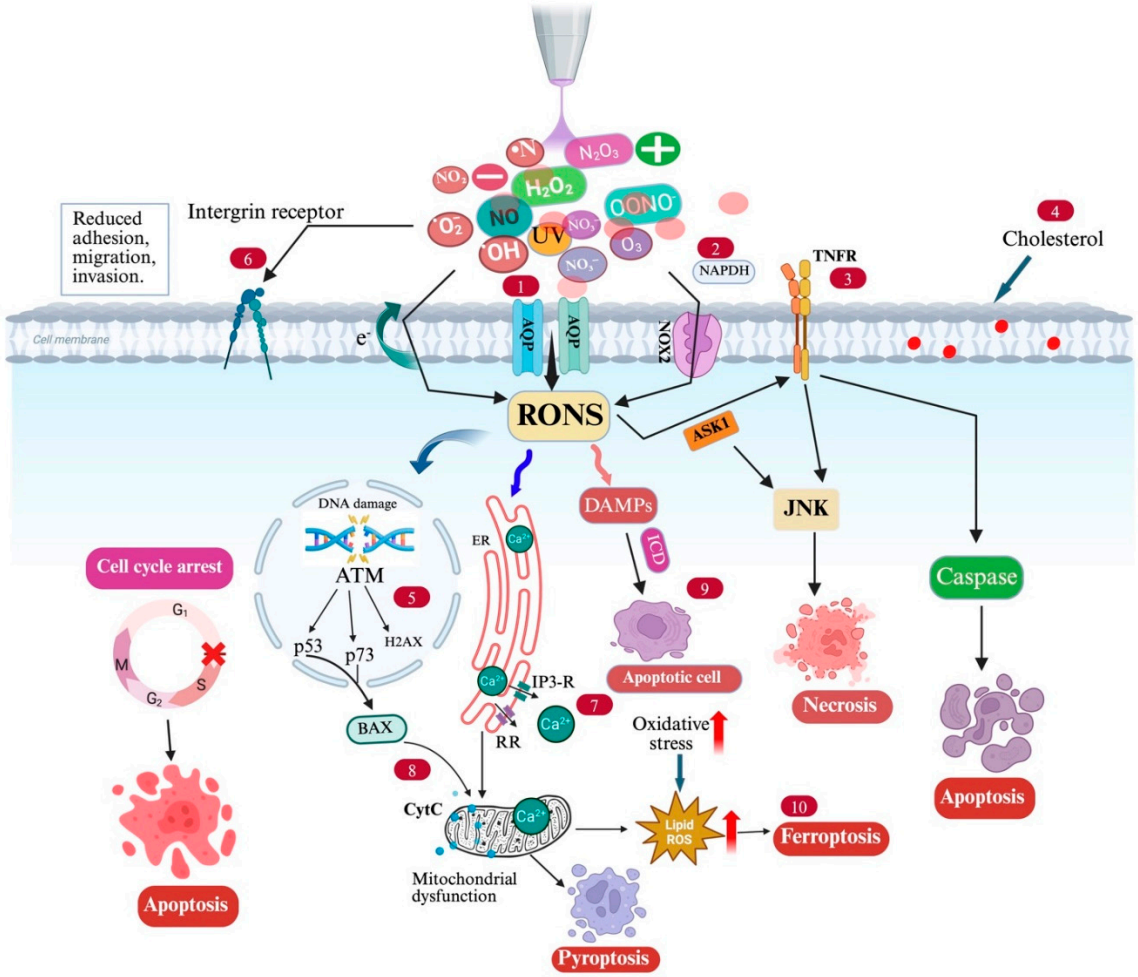

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Figure 2. Molecular pathways involved in CAP-induced anticancer responses. (1) RONS enter cells primarily through aquaporins (AQPs), which are frequently overexpressed in cancers. Direct diffusion across the phospholipid bilayer is relatively small [31]. (2) NADPH oxidase 2 (NOX2) catalyzes the NADPH-dependent single-electron reduction of oxygen, generating superoxide anions [32]. (3) Reactive species generated by CAP can stimulate macrophages, resulting in enhanced TNF-*α*–dependent activation of NF-κB and upregulation of proinflammatory genes linked to tumour development [33]. (4) Free radical-induced lipid peroxidation creates membrane pores that allow reactive species to enter cells. This phenomenon may be exacerbated in cancer cells because of lower cholesterol levels, which typically preserve membrane fluidity and stability [23]. (5) CAP exposure leads to DNA double-strand breaks and activates a DNA damage response pathway involving p53, p73, H2AX, and ATM, likely secondary to CAP-induced apoptosis rather than direct DNA damage [34]. (6) Decreased cell adhesion, migration, and invasion after CAP treatment may result from the downregulation of integrin expression [35]. (7) Increased intracellular RONS disrupt calcium signaling, for example by interacting with inositol trisphosphate(IP3-R) and ryanodine receptors(RR), resulting in elevated calcium influx into the cytosol[35]. (8) RONS-induced endoplasmic reticulum stress triggers calcium transfer to mitochondria, leading to membrane depolarization and activation of mitochondria-dependent apoptosis [36]. (9) the induction of immunogenic cell death (ICD) in plasma-treated cancer cells [37]. (10) CAP-generated intracellular ROS activates oxidative stress–related genes, impairs mitochondrial function, and modulates FSP1/NRF2 expression, leading to elevated lipid ROS levels that trigger ferroptosis [38]. Figure created by BioRender.com.

Evidence suggests that CAP can target several types of cancer and may be employed either as an independent treatment or together with conventional therapies to improve overall therapeutic efficacy. Both in vitro and in vivo studies have extensively examined the anticancer effects of CAP. The majority of in vitro assays utilize human or murine cell lines, while in vivo studies are essential for translating CAP into a clinical setting for cancer treatment [39]. In a project investigating Non-Thermal Plasma for pancreatic cancer treatment (Figure 3a), researchers observed that CAP application resulted in reduced tumor growth in a murine model [40]. The promise of CAP as a therapy for gliomas/GBM lies in its multi-faceted action: Research by Chen et al. shows the μCAP jet can penetrate the skull to treat glioblastoma (Figure 3b), Over two days, tumors in untreated (control) animals grew by nearly 600%. In contrast, tumors treated with He μCAP shrank by approximately 50% below their starting size. These results show that He μCAP treatment can significantly inhibit tumor growth [41]. CAP has also proven effective against lung cancer, where longer exposure times lead to a marked increase in cancer cell death (Figure 3c) [42]. Non-thermal plasma exerts antitumor effects in head and neck cancers (Figure 3d), by elevating intracellular reactive oxygen species, leading to a reduction in tumor size [43]. CAP is a promising minimally invasive therapy for skin cancer, utilizing oxidative stress to selectively induce tumor cell apoptosis. In the study by Liu et al. (2024), an A431 cell xenograft model in nude mice was established. Their results showed that tumor volume differed significantly between the untreated and CAP-treated groups (Figure 3e) [44].

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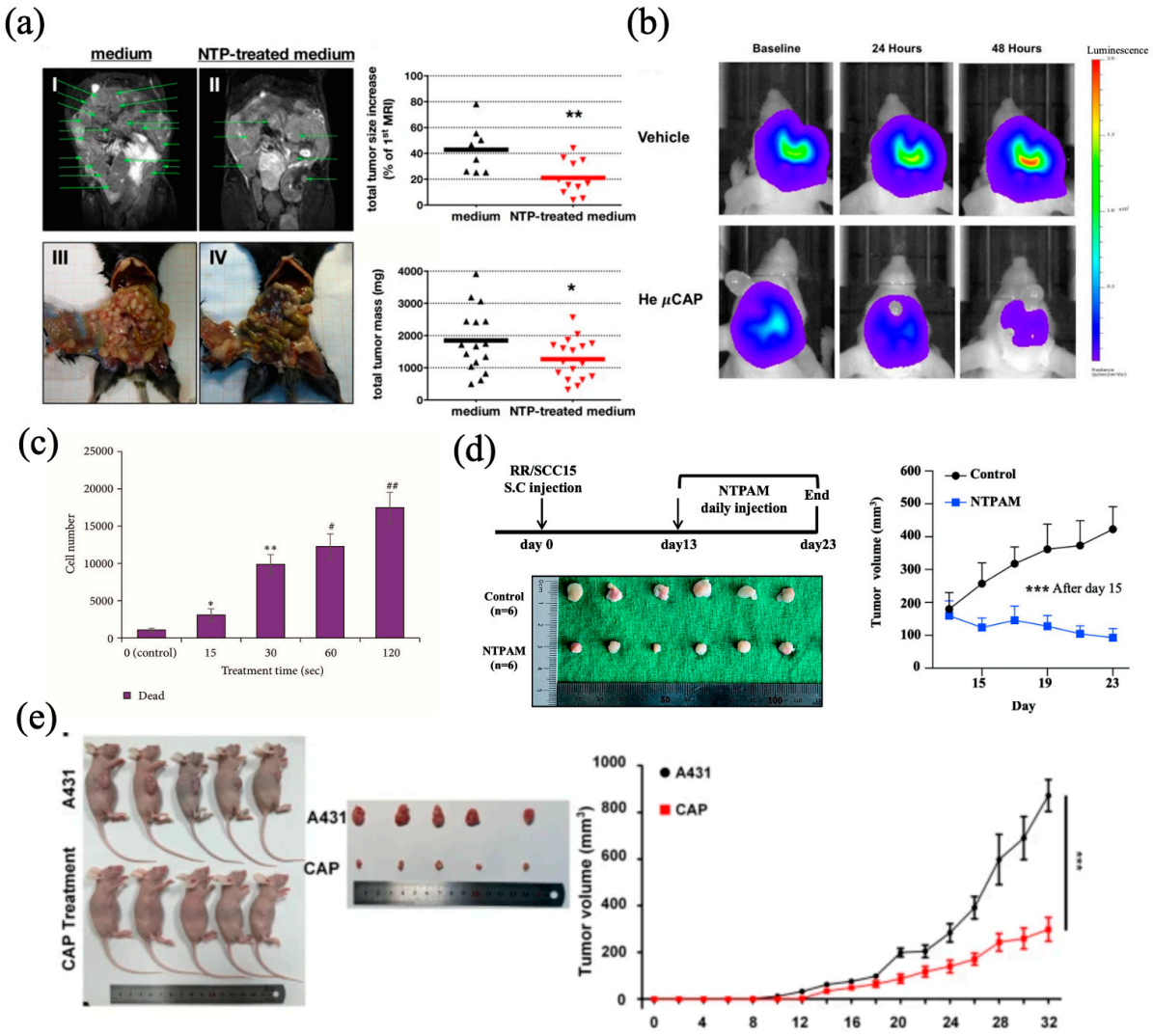

Figure 3. CAP mechanisms across different cancers: (a) Pancreatic cancer: reduction in tumor growth. Reproduced with permission. [40] Copyright 2017, Springer Nature. (b) Bioluminescence images showing tumor size pre- and post-treatment. Reproduced with permission.[41] Copyright 2017,Wiley. (c) Lung cancer: Induction of cell death post treatment. Reproduced with permission. [42] Copyright 2017, Wiley. (d) Head & Neck cancer: reduction in tumor size. Reproduced with permission. [43] Copyright 2025, Antioxidants & Redox Signaling. (e) Skin cancer: Comparison of tumor volumes in control versus CAP-treated cohorts. Reproduced with permission. [44] Copyright 2024, Springer Nature.

A selective effect of broadband electromagnetic radiation from atmospheric pressure helium high-frequency discharge plasma (generated within a closed dielectric volume) has been revealed. The effect manifested itself in growth inhibition and induction of in vitro cell death of glioblastoma (U87MG line) and melanoma (B16F10 line) cancer cells due to a newly-discovered mechanism: compromise of cell membrane integrity due to electromagnetic field–induced membrane vibrations [45,46]. This electromagnetic effect on cells turned out to be stronger than the solely chemical effect of antitumor drugs, and the effect is possible even through material barriers (glass, quartz, plastic and distilled water) and at some distance from the plasma source. The potential of such electromagnetic effect on cancer cells lies in the fact that it will allow one to overcome the biological limitations of the effectiveness of the antitumor effect of chemical drugs, or to complement and enhance their effect. Further experiments showed that the electromagnetic effect works even when the thermal factor on cells is minimized; it is most strongly observed in a gas-discharge system with a single-point potential electrode concentrating the electric field intensity [45], and the effective

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distance of the long-range action on cells can be increased several times (up to several centimeters) using a focusing electrode [46]. The results can potentially be implemented in medicine and can form the basis for a technology for remote non-ionizing and non-invasive electromagnetic treatment of tumors (such as breast cancer or glioblastoma) located deep in the thickness of organs and tissues.

3. Application of CAP in Breast Cancer: In Vitro Studies

CAP is increasingly studied for biomedical applications given its capacity to regulate oxidative stress, modify cell signaling, and influence both microbial and cellular functions [47]. These abilities make CAP a developing novel therapeutic approach for targeting breast cancer stem cells (BCSCs), which are key drivers of therapy resistance, tumor relapse, and metastasis [48]. Researchers employ two main CAP strategies: direct application of the plasma (direct CAP) and indirect application via plasma-treated liquids (indirect CAP), such as plasma-activated water (PAW), in both cell-based and animal studies [49,50]. Unlike conventional treatments, CAP-generated RONS can further elevate oxidative stress in cancer cells with already disrupted redox balance, while leaving healthy tissues largely uneffected because of their low baseline RONS levels, leading to selective cancerous cell damage and death [51,52].

Breast cancer frequently exhibits treatment resistance, with the incidence and underlying mechanisms varying across molecular subtypes [53]. Modern breast cancer therapy is centered on receptor-based targeting, including endocrine and anti-HER2 treatments, within the framework of personalized medicine. In contrast, TNBC exhibits a comparably high incidence of drug resistance relative to Luminal A, Luminal B, and HER2-enriched subtypes [54]. In this case, BCSCs are more responsive to CAP than normal cells in the breast tissue [55,56]. CAP is effective against TNBC, significantly decreasing the viability of BCSCs and downregulating key stemness markers such as $CD44^{+}/CD24^{-}$ and ALDH1 [57,58]. The significant reduction in cell viability prompted researchers to investigate cytoskeletal alterations following DBD-CAP exposure. Notably, a strong dose of cold plasma proved highly toxic to aggressive Hs578T breast cancer cells, with cytotoxicity reaching 70-90% after a 6-minute exposure (Figure 4a) [59]. Lee et al. [60] revealed that CAP can restore tamoxifen sensitivity in MCF-7/TamR cells by regulating resistance-related genes. They reported opposite expression trends of MX1 (upregulated) and HOXC6 (downregulated) (Figure 4b) after CAP treatment, further confirmed by Western blot. Xiang et al. [57] reported that apoptosis induced by Plasma-activated medium (PAM) was assessed using annexin V-FITC, showing a noticeable increase in apoptotic response compared with non-tumorigenic cells, while MCF7 (Figure 4c) exhibited only a slight elevation. These results indicate that PAM promotes apoptosis with selectivity toward breast cancer cells, highlighting its potential as a targeted therapeutic approach. Kim et al. found that atmospheric pressure plasma significantly inhibited breast cancer cell migration. As shown in the 2D trajectory plot (Figure 4d), APP-treated cells exhibited confined and shortened movement paths with markedly reduced motility compared with the control group, indicating a strong suppressive effect on migratory behavior [61]. Together, these results demonstrate CAP's potent and selective action against TNBC tumors and their aggressive BCSC populations through ROS-mediated pathway disruption. Tri-CAP exerts its effects by concurrently disrupting glycolysis, redox homeostasis, and the AKT/mTOR/HIF-1α pathway, inducing 75-98% apoptosis in resistant cancer cells [62], while CAP-treated media (e.g., DMEM, Opti-MEM) downregulate metastasis-related genes (VEGF, MMP9) and inhibit BCSC self-renewal [63]. Additionally, CAP overcomes chemoresistance by suppressing drug-efflux pumps for example ABCG2 and downregulating hypoxia-inducible factor-1α (HIF-1α) [64]. These effects position CAP as a valuable adjunct therapy; However, further research is needed to establish optimal treatment protocols and validate its clinical applications [48,62].

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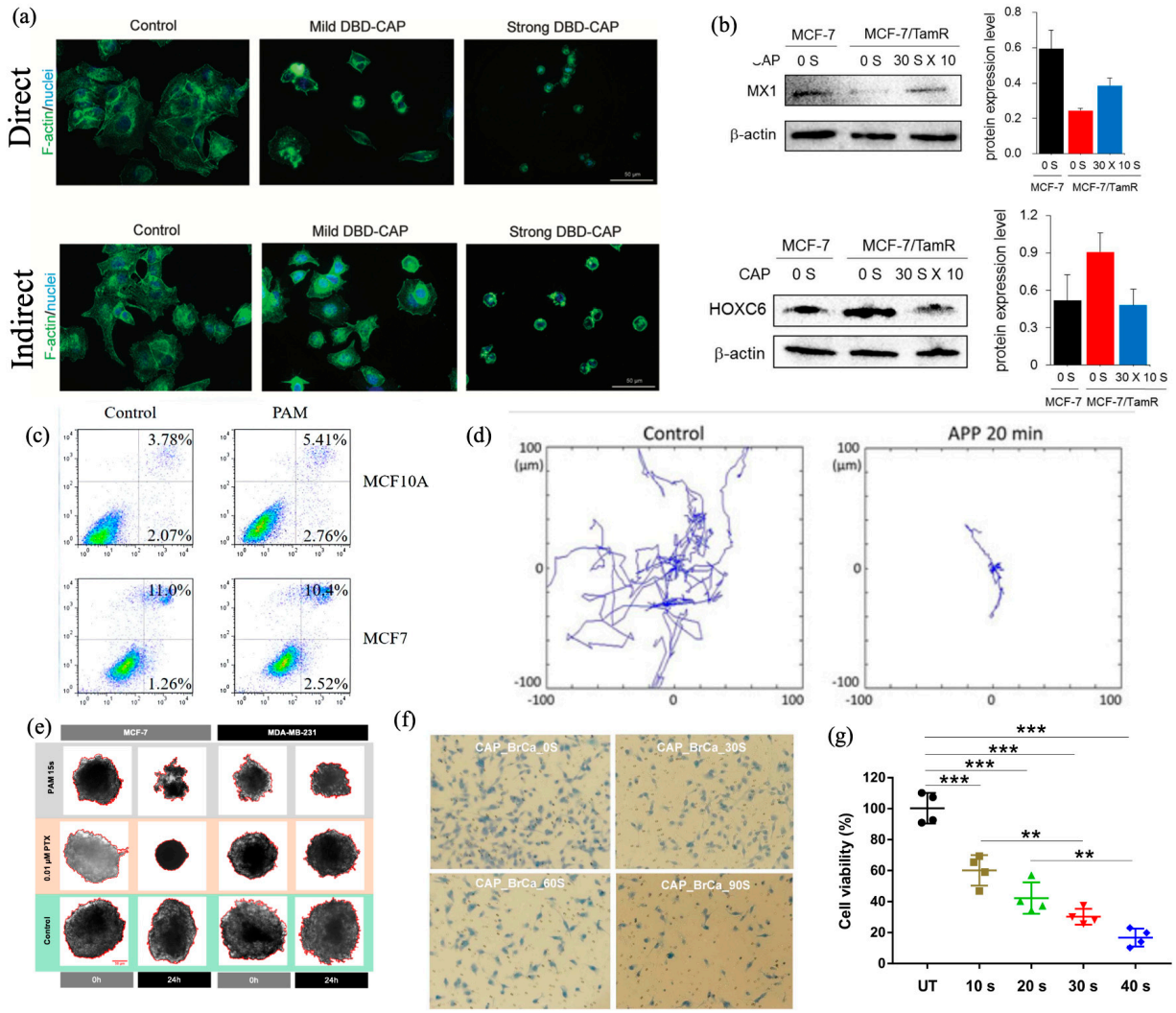

Figure 4. In Vitro CAP Treatment for Breast Cancer. (a) Cell structure (F-actin/nuclei) after 2-minute CAP treatment. Reproduced with permission. [59] Copyright 2021, Frontiers. (b Western blotting was performed to examine the expression levels of MX1 and HOXC6 proteins in CAP-exposed MCF-7/TamR cells. Reproduced with permission. [60] Copyright 2017, Elsevier. (c) Apoptosis was measured in MCF10A and non-TNBC cells after treatment with PAM. Reproduced with permission. [57] Copyright 2018, Elsevier. (d) Cell migration trajectories before and following CAP treatment. Reproduced with permission. [61] Copyright 2021, Frontiers. (e) MCF-7 and MDA-MB-231 spheroids after treatment. Reproduced from reference [65]. Copyright 2022, MDPI. (f) CAP treatment reduces invasion of breast cancer cells in a time-varying method. Reproduced with permission.[66] Copyright 2013, PLOS ONE. (g) Time-dependent effect of aCAP on 4T1 cell viability. Reproduced with permission. [67] Copyright 2021, American Association for the Advancement of Science.

Beyond its direct cytotoxicity, CAP emerges as a novel tool to selectively induce immunogenic cell death (ICD), thereby reactivating anti-tumor immunity within the suppressed breast tumor microenvironment [68]. Importantly, CAP-induced ICD enhances dendritic cell maturation and promotes antigen cross-presentation, strengthening cytotoxic T-cell responses [69]. For example, nanomaterial-enabled strategies combining immune checkpoint inhibition and photodynamic therapy have demonstrated improved antitumor immune responses and tumor suppression in TNBC models [70]. It reactivates the immune response inside the tumor, which is inhibited by cancer cells [71]. Antitumor immune responses can be activated through ICD, which induces the release of DAMPs such as ATP, calreticulin (CRT), and HMGB1 [72–74]. In this context, CAP has emerged as a novel physical modality capable of inducing ICD in breast cancer cells. CAP exerts its biological effects primarily through RONS, which quickly react with important cellular components such as

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proteins and lipids [75]. Endoplasmic reticulum (ER) stress, triggered by RONS, generates DAMPs during immunogenic cell death [76]. During this process, stressed tumor cells present CRT on their surface and sequentially release DAMPs, including ATP during apoptosis and HMGB1 [77]. ICD is characterized by the surface exposure of CRT and the release of HMGB1 into the extracellular environment [78]. Beyond classical DAMP exposure, Plasma-induced oxidative modification of tumor antigens may enhance antigen presentation and adaptive immune activation [21]. PAM not only functions as a monotherapy but can also amplify the effect of other drugs. Mihai et al., for instance, showed that pairing PAM with paclitaxel (PTX) significantly increased its potency against breast cancer cells (Figure 4e) [65]. The PAM and PTX combination enhanced anti-cancer efficacy while lowering the required PTX dose and its side effects. This positions PAM as a promising therapeutic strategy and opens new possibilities for plasma-based cancer treatments. By similarly functioning as an ICD inducer, CAP presents a promising candidate for such combination strategies in breast cancer. This inhibition of metastasis is associated with decreased MMP-2 and MMP-9 activity and alterations in ERK and p38 MAPK signaling pathways [79,80]. Therefore, CAP's role extends beyond direct cytotoxicity (such as its documented anti-metastatic effects on motility) [81,82], positioning CAP as a potentially effective method of causing ICD in the treatment of breast cancer. Apoptosis in tumor cells is induced by CAP, which also suppresses essential metastatic processes such as cell migration and invasion [83]. In breast cancer cells, CAP disrupts the cytoskeletal structure and suppresses Rho GTPase signaling, both of which are key regulators of cellular migration and invasion, thereby impairing metastatic potential [84]. The influence of CAP on metastatic breast cancer cell migration was examined using a transwell assay, revealing a substantial reduction in both migration and invasion following CAP treatment (Figure 4f) [66]. Furthermore, the ROS generated by CAP destabilizes major oncogenic pathways, including PI3K/AKT and MAPK, which are frequently dysregulated in aggressive forms of breast cancer [81,84]. Notably, CAP mainly affects cancer cells while leaving normal tissues largely unharmed, thanks to differences in their cell membrane structures and their capacities to manage reactive oxygen species [85]. One study showed that CAP's effects on various breast cancer subtypes and found that it significantly reduces cancer cell invasiveness by lowering CD44 levels, a key adhesion molecule, in both ER-positive and ER-negative models. They also reported that CAP regulates MMP-1, MT1-MMP, and uPA in a receptor-dependent manner, lowering their expression in ER-negative cells while raising it in ER-positive cells. Research has shown that 4T1 breast cancer cells undergo significant death following aCAP application, and longer exposure correlates with greater cell killing (Figure 4g) [67]. Additionally, CAP modulates inflammatory cytokines by decreasing IL-6 and IL-8 expression in ER-negative breast cancer cells but increasing their levels in ER-positive cells[59]. Similar anti-invasive effects have been reported in thyroid papillary carcinoma and hepatocellular carcinoma, where CAP disrupts the cytoskeleton and reduces protease activity [86,87]. Disruption of signaling pathways including STAT3, MAPK, and NF-κB by RONS contributes to decreased cell adhesion and reduced invasive behavior [88,89]. Additionally, CAP appears to preferentially affect cancer cells likely due to variations in membrane cholesterol that influence ROS penetration[90]. These various molecular effects imply that CAP modifies the invasive properties of cancer cells in addition to inducing death..

4. Application of CAP in Breast Cancer: In Vivo Studies

Investigations into direct CAP treatment have revealed its dual efficacy in breast cancer models, effectively inhibiting tumor progression and reducing the risk of recurrence [91]. For indirect CAP treatment, researchers use a PAM. This liquid, infused with reactive molecules from a plasma source, can be applied directly in in vivo studies to explore systemic anti-tumor effects [92]. CAP can induce ICD and stimulate T-cell–mediated antitumor immunity. In recent studies, the combined aPD-1@CAP-gel therapy outperformed single-agent gels, demonstrating the strongest tumor growth control. [93]. Angiogenesis within tumors, driven by regulators such as HIF-1α, PDGF, and VEGF, plays a key role in supporting cancer growth and metastatic dissemination [94]. These molecules contribute to the formation of irregular and malfunctioning blood vessels, which hinder efficient drug

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delivery and foster an immunosuppressive environment within the tumor [95]. Anti-angiogenic approaches seek to counter these harmful effects by utilizing RONS to block blood vessel formation. RONS promote the breakdown of HIF-1α and reduce VEGF production, which suppresses the growth of endothelial cells and helps restore normal blood vessel function [96]. CAP treatment suppressed both tumor cell proliferation and angiogenesis, reducing blood supply (Figure 5a) compared to the highly proliferative control [97]. Upregulation of drug efflux pumps like P-glycoprotein (P-gp) caused by aberrant tumor vasculature can contribute to chemoresistance, making vascular normalization crucial for improving drug delivery within tumors [98,99]. Also, by restoring dendritic cell maturation and boosting T-cell infiltration, vascular normalization can reverse VEGF-driven immunosuppression and increase the effectiveness of immunotherapies [100]. Anti-angiogenic treatments, however, may play a dual role in some situations, inhibiting tumor growth while also possibly accelerating tumor progression [94]. Consequently, thorough assessment and further studies are needed to enhance their clinical use [101]. In breast cancer models, resistance has been more effectively overcome through the combined application of anti-angiogenic therapy and immunomodulatory drugs such as PD-1 inhibitors [102].

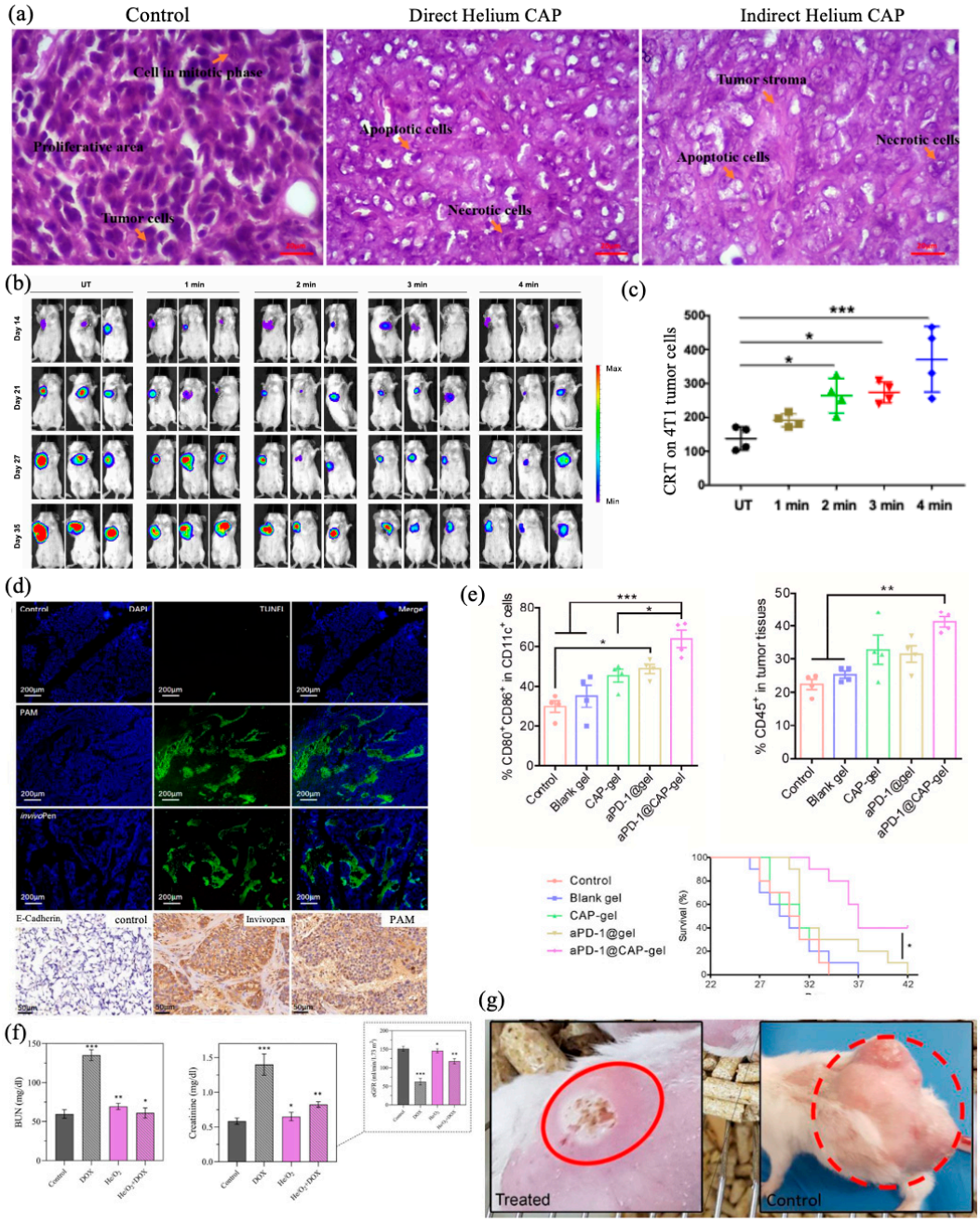

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Figure 5. In vivo CAP treatment for breast cancer. (a) Tumor histology (H&E stain) by experimental group. Reproduced with permission. [97] Copyright 2025, Nature. (b) Bioluminescence imaging of metastatic 4T1 tumors post-resection (day 14). Shown are three representative mice/group. Reproduced with permission. [67] Copyright 2021, American Association for the Advancement of Science. (c) CRT levels on the remaining 4T1 cells were quantified after aCAP treatment. Reproduced with permission.[67] Copyright 2021, American Association for the Advancement of Science. (d) Analysis of tumor cell apoptosis by TUNEL assay. & Tumor migratory potential assessed via E-Cadherin immunohistochemistry. Reproduced with permission. [103] Copyright 2020, Ivyspring International Publisher. (e) Dendritic cell maturation ($CD80^+CD86^+$ among $CD11c^+$ cells), overall immune cells ($CD45^+$), and survival outcomes in TNBC-bearing mice. Reproduced with permission. [93] Copyright 2023, Elsevier. (f) blood urea nitrogen, and creatinine with eGFR. Reproduced with permission. [104] Copyright 2023, Elsevier. (g) Studies in murine breast cancer models have confirmed the significant therapeutic efficacy of plasma. Reproduced with permission. [102] Copyright 2020, Elsevier.

CAP fights cancer by activating the immune system against tumors through several key mechanisms. Growing evidence indicates that CAP enhances anti-tumor immunity via several distinct mechanisms [105]. Evidence also suggests that a strong antitumor immune response can be induced by aCAP, aiding in the removal of residual microtumors and reducing recurrence. Animal studies further confirm these effects, showing that treatment leads to inhibited tumor growth and longer survival in mice (Figure 5b) [67]. CAP treatment induced ICD in vivo, as evidenced by the increased expression of the DAMP, CRT in residual tumor tissue (Figure 5c) [67]. Similarly, CAP treatment of dermal fibroblasts increases the expression of angiogenin, MCP-1, MMP-9, tissue inhibitor of metalloproteinases-1 (TIMP-1), uPA, and VEGF [106]. After exposure to CAP, endothelial cells, which are especially sensitive to it, exhibit increased levels of angiopoietin-2, amphiregulin, FGF-2, and important receptors such as fibroblast growth factor receptor 1 (FGFR1) and VEGF receptor 1 (VEGFR1) [107].

CAP treatment primarily induced apoptotic tumor cell death [108]. Another study reported comparable efficacy between PAM and in vivoPen treatments, by inducing tumor cell apoptosis and inhibiting cell migration. Analysis of the treated samples confirmed a high level of apoptotic cell death (Figure 5d) [103]. A reduction in E-cadherin expression, a key adhesion molecule and EMT marker, was observed following plasma treatment [109]. E-cadherin helps suppress tumor migration, showing a strong increase in tumors treated with either PAM or invivoPen, according to our immunohistochemistry analysis (Figure 5d) [103]. This CAP-induced exposure of CRT on tumor cells, in turn, CAP-gel treatment induced ICD in tumor cells in vivo, leading to subsequent dendritic cell maturation and immune activation. They also notice that after the application of aPD1@CAP-gels, The overall numbers of matured of DCs ($CD80^+CD86^+$ in $CD11c^+$ and Immune cells ($CD45^+$) were increased in vivo [93]. Mice treated with aPD-1@CAP-gels showed significant inhibition of tumor growth, correlating with a significant extension of overall survival (Figure 5e) [93]. There are some results that collectively establish that CAP exerts cytotoxic and tumor-growth-inhibitory effects in diverse breast cancer cell lines and in vivo [97]. To assess possible treatment-related toxicity, biochemical markers such as blood urea nitrogen (BUN), creatinine, and estimated glomerular filtration rate (eGFR) were analyzed. He/O_2-PAM treatment significantly reduced DOX-induced (Figure 5f) elevations in BUN and serum creatinine. When administered alone, He/O_2-PAM did not significantly influence renal biomarkers relative to untreated controls, indicating that it does not exhibit inherent nephrotoxic effects [104]. Animal model research has shown the considerable therapeutic potential of plasma in oncology. Using a floating electrode DBD system in a murine breast cancer model, Adil et al. found the potent anti-tumor efficacy of CAP (Figure 5g) [102]. The treatment significantly inhibited cancer cell proliferation in vitro, an effect that translated in vivo to a substantial reduction in tumorigenesis. These results demonstrate the encouraging therapeutic potential of CAP for breast cancer.

Additionally, Increased caspase 3/7 activity and DNA fragmentation found using TUNEL tests indicate that CAP caused apoptosis [110]. CAP effectively blocks VEGF-driven movement and tube

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formation in endothelial cells, which are vital steps in forming new blood vessels (Figure 6). These inhibitory effects are comparable to those seen with standard anti-angiogenic drugs like bevacizumab. However, CAP offers the advantage of avoiding the widespread side effects and resistance issues often associated with conventional treatments, suggesting it could be a safer and more efficient option as well [99,100]. In addition to its direct actions on endothelial cells, CAP alters the tumor microenvironment by producing RONS, which specifically target tumor-associated endothelial cells. This leads to oxidative stress, DNA damage, and suppression of VEGF signaling, ultimately reducing the permeability of tumor blood vessels (Figure 6) [111]. By oxidizing key ECM components such as fibronectin and hyaluronic acid, CAP weakens matrix integrity, making it more difficult for new blood vessels to form. CAP also reduces the expression of adhesion molecules, including integrins, which are required for endothelial cell migration and new vessel sprouting (Figure 6) [97,110].

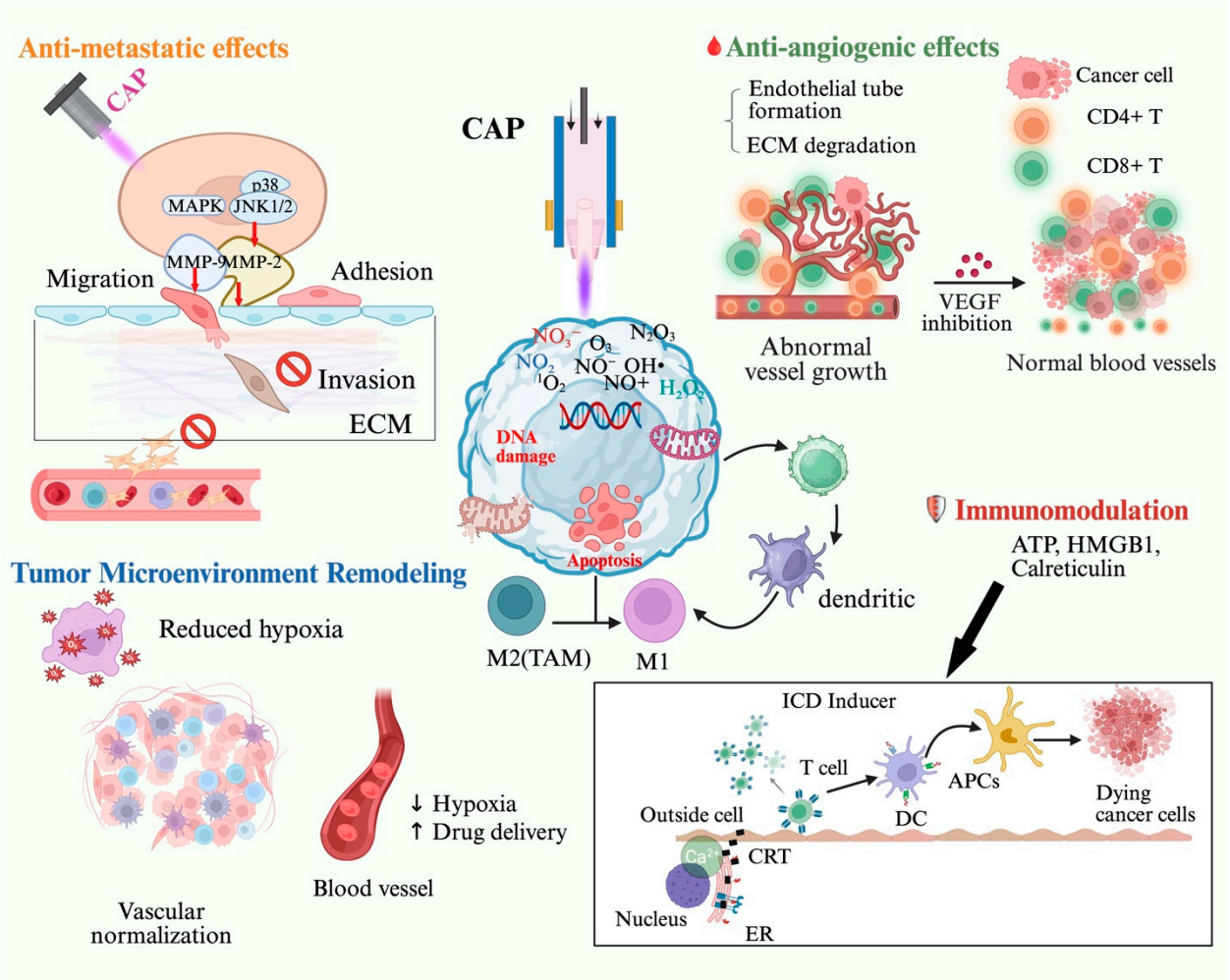

Figure 6. Graphical depiction of the multifaceted antitumor mechanisms of CAP in breast cancer. CAP-derived RONS, including NO_2^-, NO_3^-, and H_2O_2, enter cancer cells and trigger intracellular oxidative stress, resulting in cell death, mitochondrial malfunction, and damage to DNA. CAP suppresses cell migration by downregulating metastasis-associated factors such as MMP-2, MMP-9, and EMT markers. Additionally, CAP normalizes tumor vasculature, reduces hypoxia, and promotes tumor microenvironment remodeling. Anti-angiogenic effects are mediated through VEGF inhibition, impaired endothelial tube formation, and ECM degradation. CAP-induced ICD releases DAMPs, including ATP, HMGB1, and calreticulin, promoting dendritic cell activation and T-cell–mediated antitumor responses. Furthermore, A shift of tumor-associated macrophages toward the pro-inflammatory M1 phenotype is promoted by CAP, which supports the development of a more immunostimulatory tumor microenvironment. Figure created by BioRender.com.

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In vivo tumor size measurements taken before and after treatment were used to evaluate the direct and indirect effects of argon and helium plasma. The findings demonstrated that whereas CAP medication prevented the growth of breast tumors in all treated groups, tumor size rose in the untreated group. The largest inhibitory effects were observed in the direct argon and helium CAP groups. Tumor size was reduced more in the direct argon plasma group than in the direct helium group, however this difference was not statistically significant. Further, compared to the control group, both therapies markedly decreased angiogenesis, blood supply, and histology tumor scores [97]. Through immunomodulatory mechanisms, vascular suppression can occur via the M1 polarization of tumor-associated macrophages (TAMs) and the production of cytokines such as TNF-α and IL-6. Through the induction of ICD, CAP stimulates the release of DAMPs including ATP and HMGB1, promoting immune cell recruitment and strengthening antitumor immunity [112]. The selective cytotoxic effects of CAP on breast cancer cells are largely mediated by RONS generated during PAM formation, including hydrogen peroxide, nitrate, nitrite, and ammonium. A significant reduction in breast cancer cell viability has been observed following PAM treatment in vitro and in vivo, with metastatic lines like MDA-MB-231 showing marked reductions in proliferation upon CAP treatment [63]. Preclinical studies further support the antiproliferative effects of CAP, showing that CAP exposure selectively induces apoptosis in cancer cells, particularly TNBC cells, which typically have elevated basal oxidative stress levels. This apoptotic response, confirmed by Annexin V/PI staining, is identified as the primary mode of cell death following CAP treatment. Notably, normal breast epithelial cells such as MCF10A exhibit minimal sensitivity to CAP, underscoring the selective cytotoxicity of CAP toward malignant cells [57].

Several studies have confirmed that CAP selectively kills cancer cells while leaving normal cells unharmed. CAP effectively destroyed metastatic breast cancer cells but spared normal mesenchymal stem cells. This selective action is thought to result from the greater antioxidant capacity present in normal cells, which helps protect them from CAP induced oxidative stress [113]. In a similar vein, Optimized plasma conditions can selectively target head and neck squamous carcinoma cells while preserving healthy tissues [114]. Moreover, Treatment with CAP or PAM has been found to stimulate immunogenic responses and induce tumor cell death in breast cancer models, potentially enhancing immune recognition and clearance of cancer cells [48,115].

5. Plasma Immunotherapy and Other Combination Therapies for Breast Cancer

To enhance treatment effectiveness and minimize the risk of resistance often seen with single-agent therapies, combination approaches have become a key strategy in modern cancer treatment [116,117]. In line with this, CAP, due to its capacity to produce RONS, has shown great promise as an adjuvant modality in various oncological contexts, including breast cancer [118,119] .By altering cellular signaling pathways, inducing immunogenic cell death, and modifying redox homeostasis, CAP can be used in conjunction with other well-established therapeutic modalities. Furthermore, The combination of CAP with conventional cancer therapies, including chemotherapy, radiotherapy, immunotherapy, and surgery, has been reported to produce synergistic effects that improve therapeutic outcomes and may counteract drug resistance [22].

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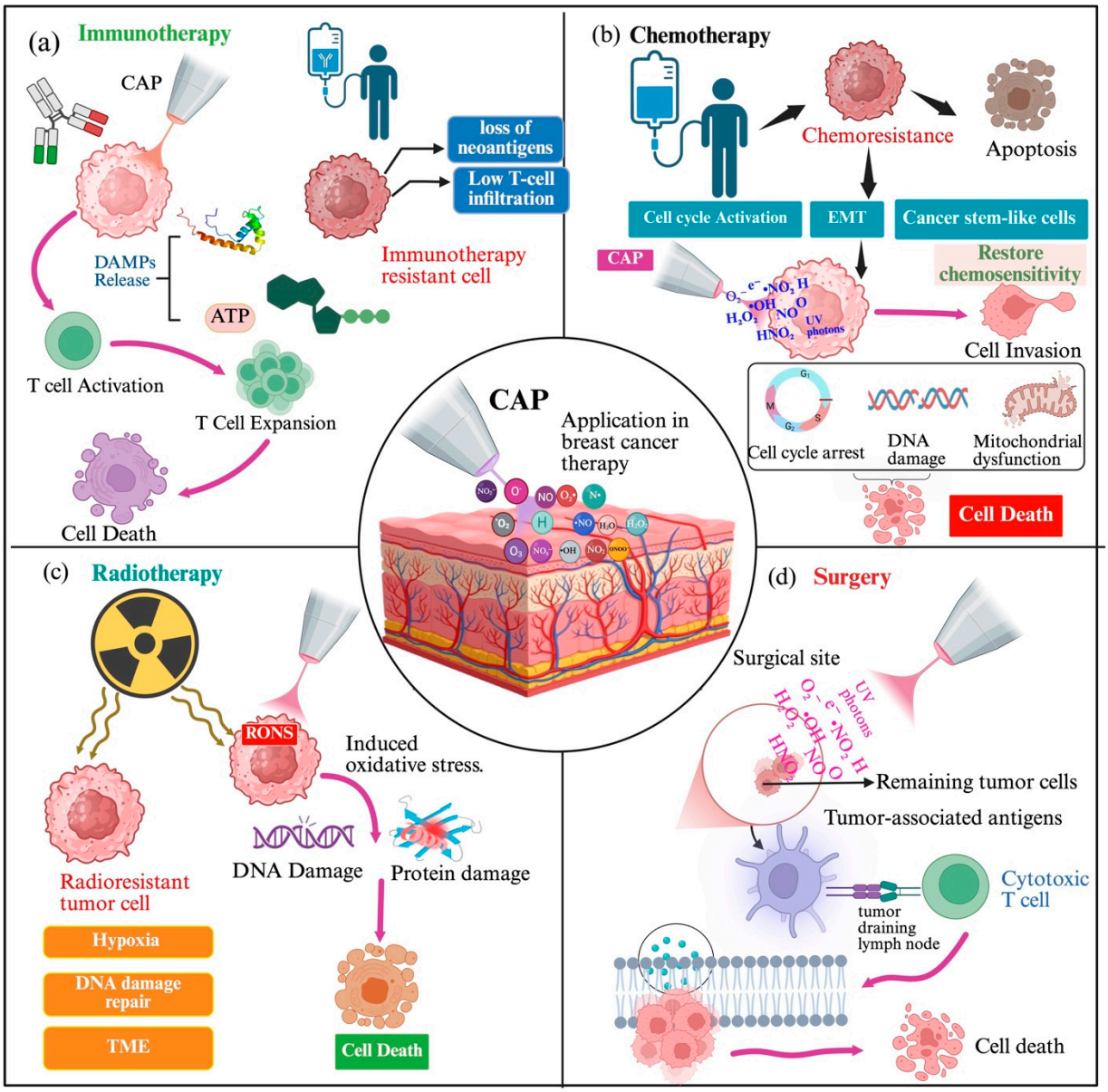

Figure 7. Illustrative summary of the synergistic effects of CAP in combination therapies for breast cancer. (a) Immunotherapy: CAP enhances tumor immunogenicity by inducing DAMP and ATP release, promoting T-cell activation and overcoming immunotherapy resistance. (b) Chemotherapy: CAP-derived RONS reverse chemoresistance by inhibiting EMT and cancer stem-like traits, leading to DNA damage and cell death. (c) Radiotherapy: CAP amplifies radiation-induced oxidative stress, impairs DNA repair, and sensitizes radioresistant tumor cells to cell death. (d) Surgery: CAP eliminates residual tumor cells after surgery and stimulates cytotoxic T-cell–mediated antitumor immunity, reducing recurrence. Figure created by BioRender.com.

5.1. CAP and Chemotherapy: Synergistic Effects on Overcoming Drug Resistance

Chemotherapy is a cornerstone of breast cancer management, where different drug classes inhibit specific pathways vital for tumor growth and survival [120]. Food and Drug Administration (FDA) approved therapies like trastuzumab, pertuzumab, and palbociclib have successfully improved patient outcomes in certain breast cancer subtypes [121]. However, Chemotherapy resistance continues to be a significant obstacle in clinical practice. To overcome this challange, combination therapies concurrently target multiple cancer hallmarks by integrating chemotherapy

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with targeted, immunomodulatory, and epigenetic agents. This strategy is key to improving sensitivity in aggressive breast cancer subtypes [122].

The development of chemoresistance is frequently linked to increased antioxidant capacity, enabling cancer cells to tolerate oxidative stress generated by both cellular metabolism and anticancer therapies [123]. Exposure to CAP counteracts this adaptation by introducing a high flux of RONS that disrupt this finely tuned redox balance. CAP-derived RONS overwhelm intracellular antioxidant systems, leading to sustained oxidative stress in resistant cells [35,124]. Consequently, in combination with chemotherapy, CAP exhibits synergistic effects that enhance drug efficacy and help overcome multidrug resistance (MDR), a major hurdle in breast cancer treatment. Alterations in cellular redox balance caused by CAP-derived RONS can regulate drug efflux mechanisms, including P-glycoprotein [125] and Alterations in cellular redox balance caused by CAP-derived RONS can regulate drug efflux mechanisms, including P-glycoprotein [126]. Resistant tumor cells can become more responsive to chemotherapeutic agents like doxorubicin and paclitaxel following CAP exposure, which induces lipid peroxidation, mitochondrial dysfunction, and DNA damage, thereby reactivating apoptotic pathways [66,127].

The researchers findings indicate that the cap does not impair drug uptake in combination therapy treatment with Paclitaxel (Figure 8a). CAP effectively resensitizes resistant cancer cells to chemotherapy. It suppresses their aggressive growth and, when combined with Paclitaxel, leads to a significantly greater reduction in cell proliferation (Figure 8b) [128]. Indirectly, CAP can be utilized as PAM in combination with diverse chemotherapies. An added benefit of PAM is its storability, which facilitates its administration via direct tissue injection, facilitating operation, administration, and its combined use with other drugs [129]. Mihai and colleagues has found that the apoptosis of breast cancer cell was increased by PTX and PAM (Figure 8c) [130]. Preclinical models have shown that CAP, when applied before or in combination with chemotherapy, can significantly lower the required drug dose while maintaining or enhancing therapeutic efficacy, thereby potentially reducing systemic toxicity [131].

5.2. Enhanced ROS Generation via CAP and Radiotherapy

Radiotherapy stands as one of the most potent treatment modalities for a wide range of human cancers. Emerging nanoplatforms can enhance the therapeutic outcome of photodynamic therapy (PDT)-radiotherapy in breast cancer by elevating ROS levels and inducing apoptosis associated with DNA damage [132]. Ionizing radiation acts either directly or indirectly by generating free radicals, which produce ROS. As a result, cells experience oxidative stress accompanied by substantial DNA damage, including the formation of lethal double-strand breaks [133]. Treatment resistance to radiotherapy in breast cancer is a major clinical concern, as it frequently contributes to therapeutic failure and cancer relapse. It often develops as cancer cells adapt by strengthening their antioxidant defenses, benefiting from hypoxic tumor regions that limit radiation-induced ROS formation, and activating more efficient DNA damage repair mechanisms [134,135]. CAP enhances the generation of intracellular ROS, exacerbating DNA double-strand breaks [37] and impairing the repair mechanisms critical for cell survival post-irradiation [136]. In addition to direct DNA damage, CAP modulates the TME by promoting vascular normalization, reducing hypoxia, and sensitizing cancer cells to ionizing radiation [48,137]. These mechanisms culminate in improved local tumor control, radiosensitization of hypoxic tumor regions, and a potential reduction in radiation doses required for effective therapy, thus minimizing collateral damage to healthy tissues [138]. Research has shown that Non-Thermal Plasma treatment can be combined efficiently with radiotherapy to enhance the therapeutic effect against breast cancer cells (Figure 8d) [139]. Improved tumor control with fewer side effects may be possible through the integration of CAP and radiotherapy, although further preclinical and clinical investigation is required

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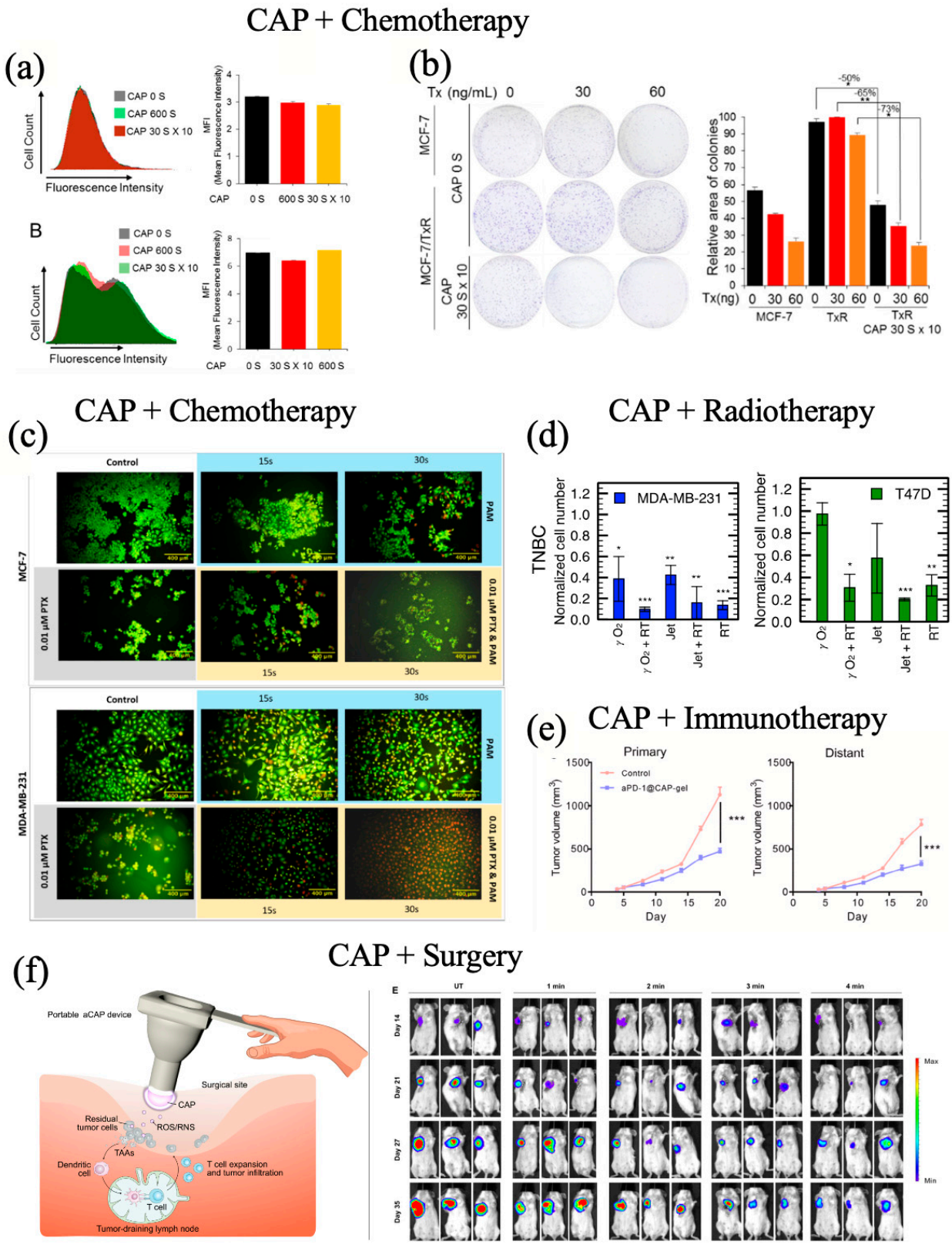

Figure 8. Representative figures illustrating the effects of combination therapy. (a) Doxorubicin and Flutax-1 uptake in MCF-7/TxR cells after CAP exposure. Reproduced with permission. [128] Copyright 2019, MDPI. (b) Changes in paclitaxel sensitivity in MCF-7 and MCF-7/TxR cells after CAP treatment. Reproduced with permission. [128] Copyright 2019, MDPI. (c) Combined paclitaxel and PAM treatment increases MCF-7 and MDA-MB-231 cell cytotoxicity more than either alone. Reproduced with permission. [130] Copyright 2022, MDPI. (d) Combined radiotherapy (4 Gy) and non-thermal plasma (10 s jet) markedly affect cancer cell viability, with differential responses across cell lines. Reproduced with permission. [139] Copyright 2020, MDPI. (e)

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Average growth kinetics of primary and distant tumors over time. Reproduced with permission. [93] Copyright 2023, Elsevier. (f) Postsurgical treatment of 4T1 tumors using a portable air-fed CAP device (day 14). Reproduced with permission. [67] Copyright 2021, American Association for the Advancement of Science.

5.3. CAP and Immunotherapy, Enhanced Immune System Targeting

Beyond chemotherapy and radiotherapy, CAP has also evolved into a powerful immunomodulatory tool in breast cancer therapy, with the potential to enhance and synergize with a wide range of immunotherapeutic strategies. RONS produced by CAP specifically target cancer cells while protecting healthy tissue, an effect particularly advantageous in aggressive subtypes such as TNBC [140]. Also, CAP has proven to be effective in targeting difficult-to-treat cancer stem cells (CSCs) by downregulating key markers, including FOXO1, ALDH1, and IL-6 [105]. A central mechanism underlying CAP's immunotherapeutic potential is its ability to induce ICD, characterized via the discharge of DAMPs and tumor-associated antigens (TAAs), like as ATP and HMGB1, from dying tumor cells [141]. These signals stimulate DCs, or dendritic cells, next, prime T cells that are cytotoxic (CTLs), initiating a robust anti-tumor immune response [93,142]. Additionally, CAP-generated RONS can induce oxidative post-translational modifications (oxPTMs) of tumor antigens, further enhancing their immunogenicity and improving T-cell recognition [143].

Beyond its effects on tumor cells, CAP remodels the TME by reprogramming immunosuppressive M2 macrophages into pro-inflammatory M1 phenotypes [144], enhancing antigen presentation and promoting T-cell infiltration [57]. Moreover, It has been shown CAP causes epigenetic alterations that improve tumor susceptibility to immune checkpoint blockade (ICB) treatments by regulating cell signaling pathways and repairing presentation of antigens [145,146]. In this context, CAP reveals favorable synergy through the use of anti-PD-1/PD-L1 antibodies and other immune checkpoint inhibitors, significantly enhancing treatment efficacy in preclinical TNBC models by increasing tumor-infiltrating lymphocytes (TILs), while lowering levels of immunosuppressive cells such as MDSCs and Tregs and simultaneously increasing $CD8^+$ T-cell activity [147]. When CAP-loaded hydrogels were combined with anti-PD-1 treatment to treat both primary and metastatic breast cancers, studies demonstrated a marked suppression of tumor growth at both sites in mice given aPD-1@CAP-gel treatment (Figure 8e) [93]. These effects not only suppress primary tumor growth but also trigger systemic cancer prevention immunity, hence lowering the chance of metastasis [148]. Enhanced CAR T-cell therapeutic activity may result from CAP-induced upregulation of MHC-I on cancer cells and alterations in the tumor microenvironment, which together improve immune detection and decrease tumor immune evasion. These effects may also reduce treatment-related toxicities, including cytokine release syndrome, and allow lower therapeutic doses [149]. One of the major limitations of CAP is its poor tissue penetration, which restricts its application to superficial tumors. However, recent technological advances, such as injectable CAP-activated hydrogels and nanoparticle-based delivery systems, now enable sustained and localized release of RONS alongside immunotherapeutic agents, improving treatment accessibility for deeper or less accessible tumor sites [150]. Both systemic immune responses and local tumor suppression have been achieved by these delivery platforms in preclinical TNBC models [151].

5.4. Intraoperative and Postoperative Applications of CAP in Breast Cancer Surgery

For most solid malignancies, surgery is still essential for effective cure and local control [152,153]. Even with improvements in the accuracy of surgery, even after tumor resection, microscopic tumor remnants and circulating tumor cells may persist [67,154–156]. Surgical trauma induces perioperative inflammation that creates a pro-tumorigenic environment, increasing the likelihood of recurrence, local relapse, and metastatic spread [157–159]. CAP can be applied during breast cancer surgery to the tumor bed or margins, targeting residual microscopic tumor cells whilst protecting normal tissue [160]. Intraoperative CAP could sometimes modulate the microenvironment of the tumor and enhance local immunological reactions [160,161]. CAP treatment at the surgical site can enhance antimicrobial defense, limit inflammation, and promote tissue healing, especially in patients

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receiving chemo- or radiotherapy [162–165]. The majority of modern CAP devices employ helium or argon, requiring bulky pressurized gas supplies that limit medical convenience [166,167]. Chen et al. investigated the use of a typical, portable aCAP device to stop the recurrence of local tumors after surgery. Their study shows that aCAP elicits a strong immunological response to tumors, preventing tumor recurrence and persistent microtumors in mice with 4T1 tumors (Figure 8f) [67]. While preclinical and early clinical studies show that CAP can be safely applied during and after surgery, standardized protocols, optimal doses, and long-term outcomes are still unclear. Further clinical studies are needed to determine CAP's optimal use and its effect on recurrence and patient outcomes in breast cancer.

Table 1. Comparative overview of CAP effects across breast cancer subtypes.

Subtype	Key Molecular Features	Sensitivity to CAP	Mechanisms of Action	Immune Modulation	Effective Combination Strategies
Normal-like (ER^+)	Estrogen receptor positive; typically low proliferation rate	Moderate [59]	Induction of apoptosis via ROS; moderate mitochondrial dysfunction[58]	CAP increases IL-6, IL-8 in ER^+ cells[59]	Chemotherapy (e.g., paclitaxel), PAM, nanoparticles[58]
$HER2^+$	HER2 amplification; high proliferation and invasiveness	High [79,80]	Suppression of MMP-2/9, inhibition of ERK, activation of p38 MAPK [79,80]	Induces ICD; affects NF-κB signaling [48,84]	Chemotherapy, anti-HER2 agents, potential synergy with radiotherapy [127]
TNBC	Lacks ER, PR, and HER2; high plasticity and stemness; often immune-cold	Very High [57,64]	Disruption of redox balance; AKT/mTOR/HIF-1α suppression; CSC targeting[62,64]	Strong ICD induction; reprograms M2 to M1 TAMs; ↑ $CD8^+$ T cells[48,86,148]	Immunotherapy (PD-1/PD-L1), chemotherapy (doxorubicin), CAR-T, PAM-hydrogel systems[148,151]
Luminal A	ER-positive, ERBB2 (HER2)–negative, gene co-expression network (GCN) is the least dissimilar from healthy breast tissue [168].	Moderate [169]	time-dependent sensitivity to CAP, characterized by apoptosis, cell-cycle arrest, and reduced viability[169].	CAP induces ICD and moderate pro-inflammatory immune activation in Luminal A (MCF-7) breast cancer via CRT-, HSP-, ATP-, and	Endocrine therapy + CDK4/6 inhibitors (Aromatase inhibitor (letrozole, anastrozole, exemestane) + palbociclib,

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				IL-6–mediated pathways[170].	ribociclib, or abemaciclib) [171].
Luminal B	More aggressive ER+ subtype, Lower progesterone receptor (PR) expression[172].	Low [173]	Inhibition of proliferation via Ki-67 suppression, disrupts G1 phase activity, time-dependent caspase-driven apoptosis, resulting in strong inhibition of tumor cell proliferation[174].	Induces ICD, upregulates antigen-presentation machinery (CRT, HSPs, MHC-I), and modulates immune checkpoint-related molecules such as PD-L1[175].	integrating endocrine therapy with CDK4/6 inhibitors, mTOR or PI3K pathway inhibitors, and HER2-targeted agents (when HER2-positive)[176].

Comparative overview of CAP effects across major breast cancer subtypes. The table highlights molecular characteristics, relative CAP sensitivity, predominant mechanisms of action, immunomodulatory effects, and potential combination strategies based on current preclinical evidence.

6. Challenges and Limitations

While CAP therapy holds significant potential for treating breast cancer, further challenges must be overcome prior to its widespread use in clinical settings. These challenges span biological, technical, and procedural aspects, including limited tissue penetration, tumor microenvironment variability, short-lived RONS, and complex interactions with the immune system. Additionally, issues with device standardization, safety protocols, and treatment costs hinder CAP's clinical translation. This section examines these limitations and ongoing efforts to overcome them, aiming to unlock CAP's full therapeutic potential.

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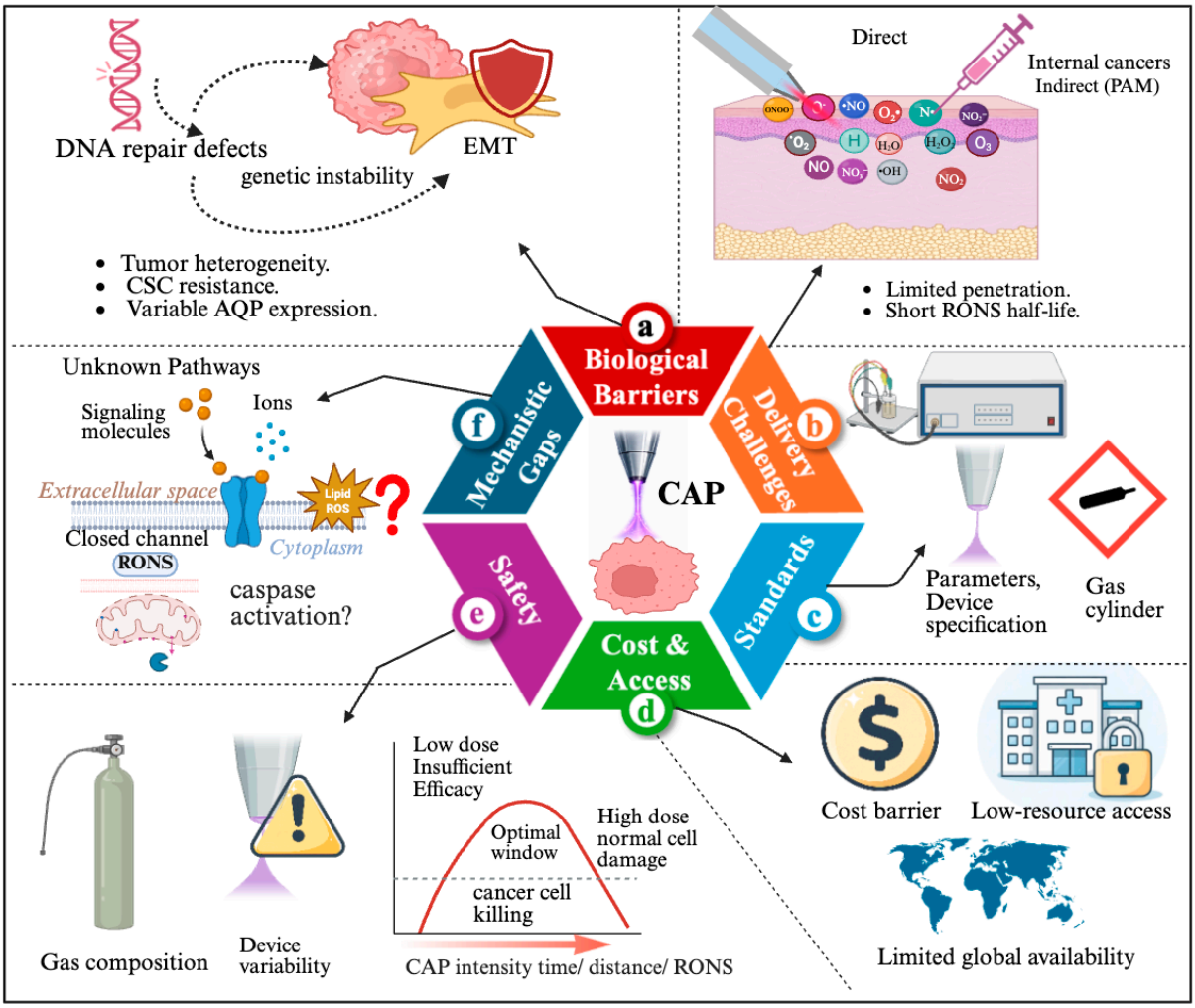

Figure 9. Major challenges limiting the clinical use of CAP in breast cancer therapy. (a) Biological barriers: Tumor heterogeneity, CSC resistance, and variable AQP expression contribute to genetic instability and epithelial, mesenchymal transition (EMT), resulting in inconsistent cellular uptake of CAP-generated RONS. (b) Delivery challenges: The limited penetration depth of CAP and the short lifetime of RONS restrict therapeutic effects primarily to superficial tissues, necessitating alternative delivery strategies such as PAM for internal tumors. (c) Standardization issues: Variations in plasma device configurations, operational parameters, and gas sources result in inconsistent RONS production and treatment reproducibility. (d) Cost and accessibility: High equipment costs, dependence on specialized gases, and infrastructure requirements limit the scalability and global availability of CAP-based therapies. (e) Safety considerations: CAP exhibits a narrow therapeutic window in which insufficient doses fail to eliminate cancer cells, while excessive exposure may induce oxidative damage to normal tissues. (f) Mechanistic gaps: Incomplete understanding of CAP-induced intracellular signaling pathways, ROS thresholds, ion transport mechanisms, and apoptotic signaling limits the optimization of CAP-mediated anticancer strategies.

6.1. Delivery Challenges

A key drawback of CAP therapy in breast cancer management is the limited tissue penetration of plasma-derived reactive species and electric fields, confining its effectiveness largely to superficial lesions [58,60,177]. To overcome this challenge, indirect CAP delivery systems have been advanced, improving their applicability for deeper tumors. These approaches utilize mediums such as CAP-activated liquids, hydrogels, tubular devices, and microneedle patches to stabilize, direct, or transport the therapeutic effects to the tumor site [91]. These carriers can penetrate deeper into breast tumor tissues while maintaining CAP's anti-cancer properties, thus extending its therapeutic effect beyond superficial lesions [178,179]. However, the instability and rapid decay of RONS in these

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formulations remain a significant challenge, limiting their consistent and effective application in breast cancer therapy [65].

Drug-loaded core-shell nanoparticles combined with CAP exposure have demonstrated synergistic effects by enhancing intracellular uptake and sustained drug release while minimizing off-target toxicity [58,180]. Nanocarrier systems based on lipid nanoemulsions have also been developed to improve paclitaxel delivery and therapeutic efficacy in breast cancer [181]. Moreover, functionalizing these nanoparticles with CAP-stimulated biomolecules may further improves targeting specificity and therapeutic outcomes in breast tumors [182]. For more effective breast cancer therapy, researchers are pursuing dual strategies to optimize CAP delivery. One approach involves encapsulating CAP or PAMs in injectable hydrogels to localize the treatment and control its release directly at the tumor. In parallel, physical methods like ultrasound and electroporation are being explored to disrupt tissue barriers, enhancing permeability and enabling these therapeutic agents to reach deeper into the tumor site [180]. Additionally, fine-tuning key CAP parameters, including voltage, frequency, exposure time, and gas composition, is essential for maximizing cancer cell targeting while minimizing harm to healthy tissues in breast cancer treatment [6,183]. Combination therapies involving CAP and conventional chemotherapeutic agents have demonstrated improved cytotoxicity in drug-resistant breast cancer models. CAP exposure can enhance the intracellular uptake of agents such as paclitaxel, enabling dose reduction while maintaining efficacy and potentially lowering systemic toxicity [65,184]. Notably, recent work has demonstrated that the transient microchannels generated by microneedle administration can substantially enhance the transdermal administration of NO generated from CAP, effectively overcoming the impedance posed by intact tissue barriers [185]. These approaches suggest a powerful adjunct role for CAP in overcoming multidrug resistance.

6.2. Biological Challenges

Several biological and technical challenges limit the scientific effectiveness of CAP in breast therapy for cancer. One primary obstacle is the heterogeneity of breast cancer subtypes, which vary in susceptibility to CAP-induced oxidative damage as a result of variations in membrane composition, antioxidant defenses, and stemness-associated traits [108,186]. Cancer stem cells (CSCs) within breast tumors are particularly resistant, possessing enhanced DNA repair and redox buffering systems that protect them from CAP-induced damage. It has been demonstrated that CAP regulates FOXO1 K48-ubiquitination, AQP3-19Y, and AQP3-5K, potentially overcoming CSC resistance [108,182]. However, these modifications are not always sufficient to fully sensitize CSCs to CAP. CAP's efficacy is also compromised by the short-lived nature of RONS, which degrade rapidly in physiological environments, reducing their bioavailability and limiting their therapeutic window[186]. Furthermore, inconsistent intracellular delivery of RONS is influenced by variations in AQP expression and membrane transport mechanisms across different breast cancer cell populations [108,182]. CAP therapy is further challenged by the immunosuppressive tumor microenvironment, where MDSCs and tumor-associated macrophages inhibit the anti-tumor immune response, thereby diminishing plasma's immunostimulatory effects [187]. KAT6A has been shown to modulate macrophage polarization and SMAD3 acetylation, affecting immune cell recruitment in triple-negative breast cancer [65,187]. CAP exposure triggers compensatory mechanisms, such as increased antioxidant enzymes and DNA repair pathways, which reduce apoptosis and limit CAP's long-term effectiveness. Additionally, dysregulated signaling channels including PI3K/AKT, NF-κB, and MAPK promote tumor longevity and stress adaptation [108]. Technical limitations, such as variability in plasma device configurations and treatment parameters, lead to inconsistent results in preclinical studies [103]. Tumor tissue heterogeneity complicates CAP delivery, necessitating targeted systems to localize and stabilize reactive species. Separately, CAP shows promise in reversing chemoresistance; for example, it enhances paclitaxel efficacy through gene modulation and redox balance, with plasma-activated media demonstrating synergistic effects, although the precise mechanisms require further elucidation [65].

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6.3. Exploring CAP-Immune System Interactions

The clinical application of CAP against breast cancer is considerably hindered by the unpredictable interaction among CAP-induced RONS and the patient's immune response. Although controlled RONS can stimulate immune activation, subtle changes in plasma conditions, dose, or delivery can invert this benefit, leading instead to immunosuppression and tumor escape [188]. The immunosuppressive TME is a crucial restriction of CAP in breast cancer treatment, which promotes M2 macrophage polarization and impedes the desired immune activation. CAP has demonstrated the ability to reprogram these cells toward an anti-tumor M1 phenotype, partially through epigenetic modulation involving KAT6A acetylation, which influences downstream immune effectors such as SMAD3 and interleukin-6 (IL-6) [189]. However, these immune effects are highly context-dependent, varying with CAP factors like gas type, exposure time, and delivery method [59,190]. RONS have a double-edged role. Moderate levels can stimulate antitumor immunity through T-cell recruitment, dendritic cell activation, and immunogenic cell death, while excessive or prolonged exposure drives oxidative stress that weakens immunological reactions and promotes immunological avoidance of tumors [191]. The proper dosage of RONS is essential, but it can differ from patient to patient, making this balance challenging. Additionally, differences in tumor subgroups, for example ER-positive versus TNBC, affect how well tumors tolerate oxidative stress and how responsive they are to immune treatments [118]. Despite the ability of CAP treatment to trigger the release of inflammatory signals like IL-6 and IL-8, these effects are often outweighed by the immunosuppressive nature of the tumor microenvironment [59]. Therefore, modern therapeutic strategies seek to reshape the tumor immune microenvironment to facilitate immune cell infiltration and strengthen the response to cancer immunotherapy [192]. Features like hypoxia, a dense extracellular matrix, and persistent inhibitory signaling act in concert to block immune cell infiltration and function [193]. In certain contexts, this imbalance may even create conditions that favor tumor progression [59]. Since differences in CAP devices, plasma sources, and treatment plans result in varying RONS production and immune responses, standardization is still a significant barrier to clinical use and reproducibility. The link between CAP exposure and particular immune outcomes over time is made more difficult by the quick production, breakdown, and bioavailability of RONS [190,194]. It is difficult to rationally optimize CAP therapy for immune benefits due to the insufficient comprehension of the fundamental mechanistic processes the intracellular pathways modulated by RONS, including NF-κB, STAT3, and IRF transcription factors [195]. The dynamic tumor-immune interactions are also not adequately replicated by current preclinical systems, especially in immunologically "cold" tumors simiar as TNBC, where the effects of CAP may be less beneficial or even detrimental [194].

6.4. Mechanistic Gaps

In breast cancer treatment, RONS levels must be controlled because excessive ROS can harm nearby healthy breast tissue, while subtherapeutic ROS may promote the survival of breast CSCs. Increased antioxidant defenses are frequently seen in CSCs and resistant breast cancer cells, which reduce CAP-induced cytotoxicity and preserve tumorigenic potential [150]. The breast TME limits CAP's effectiveness, which is characterized by hypoxia, changed immune cell profiles, and a high antioxidant capacity. These factors further complicate RONS diffusion and stability [34,187]. The polarization of tumor-associated macrophages (TAMs) from the M2 to the M1 phenotype has been linked to CAP through KAT6A-mediated acetylation, although the detailed molecular mechanisms remain unclear. This is especially significant in invasive subtypes like TNBC [187]. Even yet, CAP-induced RONS affect apoptotic regulators such BCL-2, BAX, and caspases, the precise ROS threshold for selective apoptosis of breast cancer cells is still unknown [104,150]. Although their function is still unclear, aquaporins may affect CAP sensitivity in breast cancer cells by controlling intracellular $H2O_2$ uptake [196]. Inconsistent results and low reproducibility result from the lack of standardized CAP protocols brought on by variations in plasma sources, gas types, and dosimetry. The co-administration of CAP and chemotherapeutic agents, such as doxorubicin, which is frequently used to treat breast cancer, complicates immune modulation and RONS-mediated signaling [59,104].

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The creation of long-lived RONS that maintain tumor-specific oxidative stress without causing systemic toxicity is still a significant challenge. The reproducibility and clinical translation of CAP across breast cancer populations are further restricted by subtype-specific variations, immune variability, and genetic heterogeneity [34].

6.5. Safety and Standardization of Cap Instruments and Processes

RONS, which are essential to CAP's therapeutic action in breast tumors, are produced and distributed differently as a result of inconsistent device configurations and operating parameters, which is a significant problem when using CAP to treat breast cancer [197]. Reproducibility and the creation of standardized protocols tailored for breast cancer applications are further complicated by variations in voltage, frequency, gas composition, electrode design, treatment distance, and exposure duration [180]. Research on breast cancer using PAM adds more complexity. The plasma source and environmental conditions greatly influence the physicochemical characteristics of PAM, including its composition of RONS, pH, and stability. In breast cancer cells, these sensitivities might produce variable biological effects [198]. Changes in media temperature or pH, even tiny ones, could lead to unintentional cellular stress and lower treatment effectiveness in breast cancer models [199]. Safety issues in breast cancer treatment arise from insufficient long-term in vivo data on CAP's toxicity and immunocompatibility. Though CAP targets cancer cells specifically, repeated or systemic use, particularly in conjunction with unregulated drug delivery systems like hydrogels and nanocarriers, may result in cumulative oxidative stress and side effects [143,200]. How CAP modulates immune signaling and redox homeostasis within the heterogeneous, immunosuppressive microenvironment of breast tumors, particularly in TNBC, remains a key unresolved question [59]. Clinical adoption of CAP therapy is hampered by variability in CAP devices and protocols, which results in uneven RONS production and immune responses. Inconsistent reporting of efficacy markers such as oxidative stress, tumor response, and immune activation further delays regulatory approval[49].

6.6. Cost and Availability

The clinical translation of CAP for breast cancer faces significant hurdles, including high costs from noble gas use and specialized hardware, as well as challenges in scalability, accessibility, and standardization [39,182]. The global cold plasma market was valued at approximately US$3–4 billion in 2026, with projections indicating continued growth toward US$8 billion by 2032, driven by increasing adoption in healthcare and industrial applications [201]. Scalability is made more difficult by these technological needs, especially in areas with low and middle income [169,202]. The therapeutic efficacy of PAM is compromised by the instability of its RONS, which are highly sensitive to environmental and storage conditions [65]. Reproducibility is further hampered by the absence of standardized dosimetry instruments and procedures, which makes clinical application and regulatory approval more difficult [184,203]. Particularly in healthcare systems with limited resources, the infrastructure needed for CAP therapy, such as skilled staff, specialized labs, and dependable power sources, increases the cost [203]. Furthermore, CAP therapy is a costly option that slows its widespread adoption due to the requirement for clinical validation and regulatory approval [169,204]. To overcome these obstacles, scalable delivery systems for superficial and deep-seated tumors as well as portable, affordable, and gas-efficient devices will need to be developed. To expedite clinical-grade standards and guarantee fair access to CAP technologies, researchers, industry stakeholders, and regulatory agencies must work together [202].

7. Future Directions

CAP has moved beyond early research, with ongoing clinical applications in breast cancer treatment. To make CAP safer, more effective, and clinically relevant, upcoming work needs to refine device designs and treatment parameters, deepen understanding of biological mechanisms, align

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with personalized medicine strategies, and strengthen translational studies. This section highlights the key directions needed to advance CAP toward real clinical impact.

7.1. Optimization of CAP Devices and Protocols

Although CAP demonstrates clear anticancer potential, optimizing devices and treatment protocols is critical for clinical translation. Current CAP platforms, including DBD and plasma jets, differ greatly in voltage, frequency, gas composition, treatment duration, and electrode configuration, directly influencing RONS production and distribution, the primary mediators of CAP's therapeutic effects [205,206]. This lack of standardization limits reproducibility and slows regulatory progress [180,205]. Future efforts should prioritize clinically practical systems over engineering complexity, emphasizing portability, miniaturization, programmability, and affordability for diverse clinical settings, including low-resource environments [66,186,206]. Integration of real-time dosage monitoring, biosensing feedback, and imaging- or surgery-guided delivery can enhance therapeutic precision and patient safety [207]. Hybrid systems combining direct and indirect plasma sources or integrating DBDs, plasma jets, and electromagnetic fields offer further control over tissue targeting and penetration depth [184]. In order to facilitate efficient practical application, analysis needs to concentrate on three interdependent goals: standardizing key operational parameters (e.g., voltage, gas flow, exposure time), developing real-time monitoring systems to optimize biological outcomes, and ensuring these advances are prioritized alongside device engineering to maximize reproducibility and efficacy[180,205]. Current models frequently neglect patient-specific variations in antioxidant status and immune function, key modulators of CAP therapy response in breast cancer. Measuring these factors directly could improve biomarker discovery and enable more effective personalized treatment approaches.

7.2. Mechanistic and Translational Research-Based Breast Cancer

Recent research builds on previous findings by demonstrating that CAP causes selective cytotoxicity in breast cancer cells via epigenetic mechanisms, such as histone modifications like H3K4 trimethylation and miRNA silencing [182,208]. It further contributes to tumor microenvironment reprogramming through modulation of long non-coding RNA (lncRNA) expression, including ZNRD1-AS1 [209]. These epigenetic effects suggest potential for CAP to work synergistically with epigenetic modulators such as DNMT or HDAC inhibitors. Integrating epigenetic profiling into treatment planning may also enable patient stratification and personalized CAP therapies. In both hormone receptor-positive and TNBC models, paclitaxel and PAM, a solution treated with CAP, have demonstrated synergistic effects that enhance apoptosis, reduce cell viability, and overcome drug resistance [65]. The polarization of tumor-associated macrophages toward the pro-inflammatory M1 phenotype induced by CAP contributes to enhanced innate immune responses in TNBC, in addition to its direct cytotoxic activity [59,189]. It also reverses resistance-related genes such as CEACAM1 and DAGLA, restoring chemosensitivity in paclitaxel-resistant MCF-7/TxR cells [204]. Beyond its cytotoxic properties, CAP also participates in regulating tumor immune responses and drug resistance mechanisms.

7.3. Beyond Breast Cancer and Personalized Medicine

Recent studies suggest that the therapeutic applications of CAP are expanding to various cancers beyond breast cancer. With little harm to the surrounding healthy tissue, CAP specifically causes cancer cells to undergo apoptosis, that makes it a potentially useful melanoma treatment technique, glioblastoma, and pancreatic cancer, where it disrupts cell membranes and generates RONS [177,210]. However, differences in redox buffering capacity, membrane composition, and immune profiles across tumors may affect CAP efficacy, necessitating tumor-specific optimization. CAP also modulates immune responses, reprogramming macrophages from a tumor-promoting M2 to an anti-tumor M1 phenotype, expanding its potential across cancer types [189,211]. Beyond its direct

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cytotoxicity, CAP may synergize with chemotherapy and immunotherapy. It can reverse drug resistance mechanisms, sensitizing refractory tumors to standard treatments [212,213]. CAP's ability to remodel the tumor microenvironment and influence immune dynamics supports its integration with genetic profiling, targeted agents, and immunotherapies to tailor treatments based on tumor-specific characteristics [65,214].

Although current evidence largely focuses on solid tumors, preliminary findings suggest CAP may also be applicable to hematologic malignancies, though further research is needed. Advancing CAP's clinical utility will require deeper understanding of its molecular and immunological mechanisms [215]. Integrating CAP testing with patient-derived organoids may better predict treatment response across tumor types. As the field grows, clinical-grade device development and regulatory validation will be key for safe, standardized use. Future work should tailor CAP protocols to tumor heterogeneity and immune/redox profiles while refining delivery methods and exploring combination therapies for more effective, minimally invasive cancer treatment[214].

The therapeutic efficacy of CAP varies across breast cancer subtypes, underscoring the need to tailor treatment to the molecular and genetic profiles of individual tumors [216]. This variability highlights CAP's therapeutic potential while revealing a major limitation: its effects remain unpredictable absent precision diagnostics. A one-size-fits-all approach is therefore inadequate. Future research should integrate CAP with genetic and biomarker profiling to develop truly effective personalized treatments [189].

CAP also remodels the breast TME by modulating immune responses. In particular, it reprograms TAMs, or tumor-associated macrophages. By increasing KAT6A acetylation, CAP reprograms TAMs to have an anticancer M1 phenotype instead of a protumorigenic M2 [217]. This enhances KAT6A acetylation, which reduces SMAD3 acetylation and suppresses production of protumorigenic cytokines such as IL-6 [147]. But there are still large gaps in understanding the durability and generalizability of CAP-induced macrophage repolarization. It remains unclear whether this immunomodulatory effect is sustained over time, breast cancer-specific, or consistent across patient cohorts, highlighting the need for further comparative studies to validate its therapeutic relevance and applicabilit [189,218,219].

Beyond this mechanism, CAP's broader ability to remodel the tumor immune microenvironment positions it as a promising adjuvant for enhancing antitumor immunity and synergizing with conventional therapies [220]. These immunomodulatory effects may synergize with immunotherapies to address drug-resistant or metastatic breast cancers [182]. CAP-induced modulation of immune signaling and cancer stemness holds potential to overcome therapeutic resistance and resensitize TNBC, a kind that frequently resists conventional treatments [210]. Personalized strategies in breast cancer could leverage combinations of CAP with chemotherapy, immunotherapy, or targeted agents to exploit synergistic interactions. Tailoring such combinations according to molecular signatures may improve efficacy, especially in highly heterogeneous tumors [104]. CAP's clinical success will depend on personalized approaches aligned with each patient's tumor molecular profile and immune/redox status. Current preclinical studies often overlook inter-patient variability in immune function and redox status. Neglecting these factors risks translational failure in the clinic, even for otherwise promising therapies.

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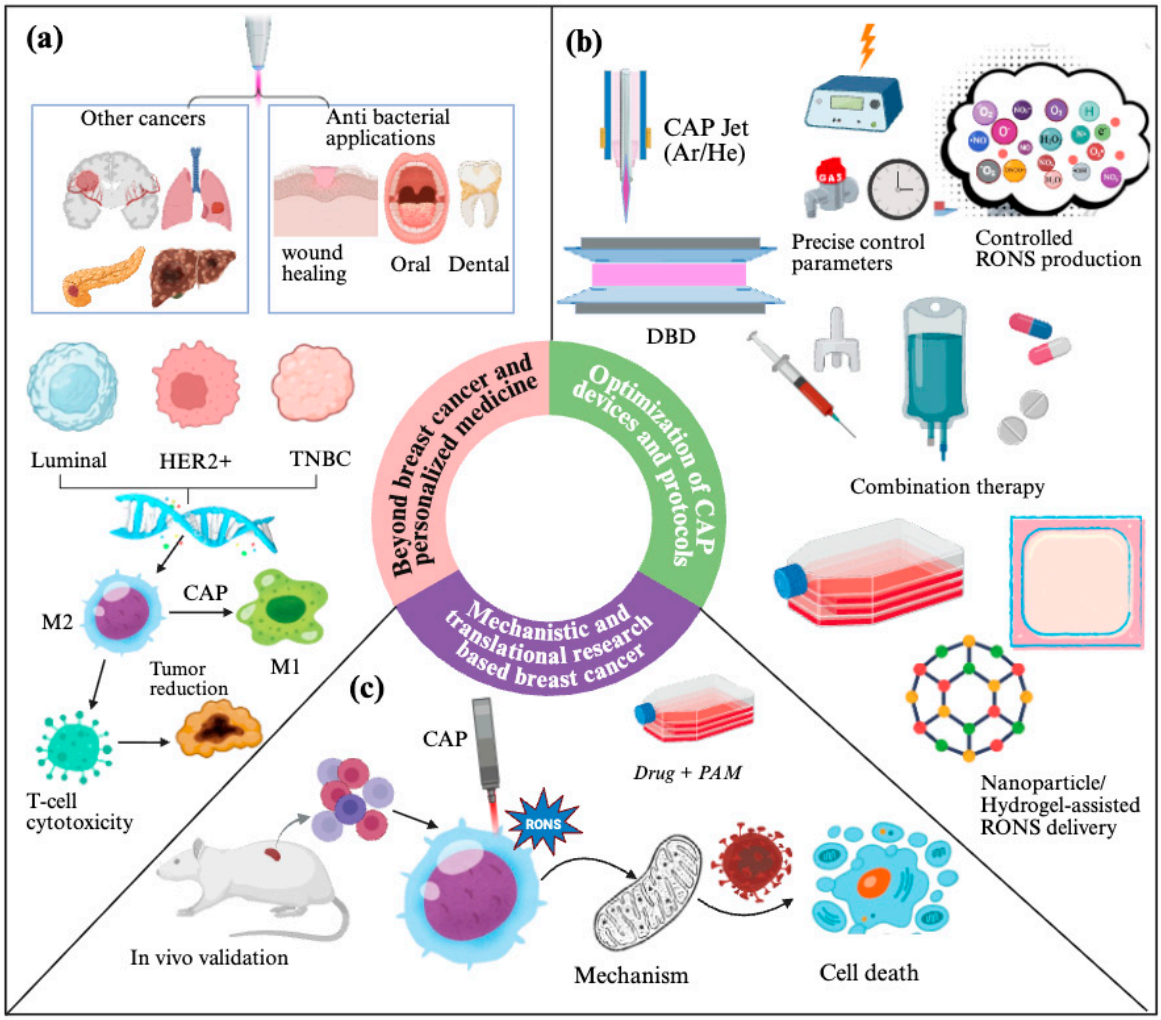

Figure 10. Future directions of CAP in breast cancer therapy. (a) Expansion of CAP toward personalized oncology across breast cancer subtypes and other medical applications, with immune modulation through macrophage repolarization and T-cell activation. (b) Optimization of CAP devices and protocols, including plasma jets and DBD systems, enabling controlled RONS generation and integration with combination therapies and advanced delivery platforms. (c) Mechanistic and translational research focusing on CAP-induced RONS signaling, mitochondrial dysfunction, and cancer cell death, supported by drug–PAM combinations and in vivo validation.

8. Conclusion

CAP is gaining increasing attention in breast cancer research because it can disrupt tumor cell survival through multiple biological pathways while showing comparatively lower toxicity toward normal cells. As discussed throughout this review, Oxidative stress, mitochondrial dysfunction, apoptosis, immunogenic cell death, and inhibition of migration, invasion, and angiogenesis are among the mechanisms responsible for the anticancer activity of CAP. The limited treatment options for aggressive breast cancer subtypes, especially TNBC, have made CAP an increasingly attractive therapeutic candidate.

Importantly, the potential of CAP extends beyond its direct cytotoxic effects. The therapeutic potential of CAP extends beyond direct anticancer activity, as it can influence the tumor microenvironment, regulate immune responses, and enhance the effectiveness of conventional treatment approaches such as chemotherapy, radiotherapy, immunotherapy, and surgery. Its ability to sensitize resistant cancer cells, alter redox balance, and affect immune signaling highlights its value

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not only as a standalone treatment but also as a supportive strategy in combination-based breast cancer therapy.

The clinical translation of CAP into routine medical practice still requires the resolution of several critical challenges. Several challenges, including poor tissue penetration, the short-lived and unstable nature of RONS, tumor heterogeneity, and the complex immune microenvironment, can influence treatment efficacy. In addition, variability in plasma devices, operating conditions, and dosimetry continues to affect reproducibility across studies. Many mechanistic questions also remain unclear, particularly regarding subtype-specific responses, long-term safety, and the balance between beneficial and harmful biological effects.

Overall, the current evidence supports CAP as a highly encouraging and rapidly developing platform in breast cancer therapy. However, its successful clinical application will depend on better standardization of devices and protocols, deeper mechanistic understanding, improved delivery systems, and more personalized treatment strategies based on tumor biology and patient-specific immune and redox profiles. With continued interdisciplinary research and careful translational validation, CAP has the potential to become an important addition to future breast cancer treatment.

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