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Review Article

The roadmap of cold atmospheric plasma for chronic diseases

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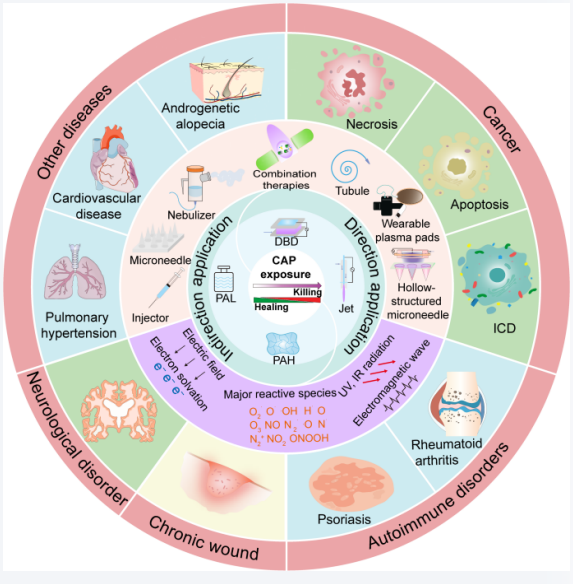
ABSTRACT: The generation and delivery techniques of cold atmospheric plasma (CAP), coupled with their innovative applications in treating various diseases, have given rise to plasma medicine as a novel therapeutic modality, offering new approaches for managing chronic diseases. CAP therapy generates a large number of reactive species that selectively modulate biological activities, yielding long-lasting effects and minimizing the risk of drug resistance, which is challenging to achieve with small molecule drugs. However, before CAP can be effectively utilized in chronic disease treatment, numerous limitations must be addressed. This review comprehensively summarizes the latest research progress in CAP-based chronic disease treatments, with a focus on the main CAP generation devices, delivery methods, and therapeutic mechanisms. Additionally, it addresses the key scientific issues in the development of plasma medicine, aiming to optimize therapeutic outcomes and provide a robust theoretical foundation and guiding principles for the effective clinical implementation of CAP.



KEYWORDS: cold atmospheric plasma, chronic disease management, plasma delivery, plasma medicine, regenerative medicine, cancer therapy

1 Introduction

According to the World Health Organization (WHO), chronic diseases are characterized by prolonged duration and generally slow progression, which are the result of a multifactorial interplay of genetic, physiological, environmental, and behavioral factors [1–3]. By 2030, chronic diseases are projected to account for 69% of all global deaths, with 80% of these deaths occurring in middle- or low-income countries [4–7]. The course of chronic disease is characterized by its persistence; therefore, its treatment typically necessitates long-term and timely therapy. However, a range of issues often hamper the therapeutic efficacy of traditional paradigms in the clinic [8, 9]. For instance, adverse medication

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experiences associated with prolonged treatment, as well as the financial burden, can significantly affect patient compliance. Additionally, some medications have poor bioavailability and require frequent dosing, thereby increasing the risk of systemic drug tolerance and the development of resistance in bacteria or tumor cells [10–13]. Furthermore, long-term drug use may result in cumulative toxicity and increase the potential for drug abuse. These limitations have driven interest in drug-free therapeutic modalities, defined as a therapeutic approach that does not rely on traditional drugs or the consumption of therapeutic agents while maintaining sustained therapeutic efficacy during treatment. Intriguingly, CAP is capable of offering a non-invasive, controllable, and drug-free platform for managing chronic diseases [14, 15]. Plasma, the fourth state of matter distinct from gas, solid, and liquid, is a fully or partly ionized gas-like medium [16, 17]. On Earth, plasma commonly manifests as lightning, and aurora borealis. Remarkably, it is estimated that plasma constitutes 99% of the visible matter in the universe. Plasma can be generated through multiple energy inputs, including thermal excitation, radiation, electromagnetic fields, and beams [18]. CAP, a typical non-thermal plasma, exists in a non-thermodynamic equilibrium state according to Maxwell-Boltzmann thermodynamic equilibrium [19]. A defining feature of CAP is that the overall temperature of the plasma, including neutrals, ions, and radicals, maintains close to room temperature, despite the presence of high-temperature electrons [20, 21]. Currently, numerous studies have conclusively demonstrated that CAP can be utilized as therapeutic agents for disease treatment or as an adjuvant in combination with other medications. This combinatorial approach not only effectively reduces the dose of medication used but also significantly enhances therapeutic outcomes [22–24]. Crucially, prolonged exposure to CAP did not induce drug resistance in either bacterial or tumor cells [25]. Given these advantages, CAP has been demonstrated to show great potential for applications in the treatment of chronic diseases that require long-term medication management.

Although considerable research and reviews have investigated the applications of CAP in sterilization, wound healing, cancer treatment, and autoimmune diseases, comprehensive reviews focusing specifically on its therapeutic potential in chronic diseases remain relatively scarce. In this review, we critically assess the strengths and limitations of current primary CAP generation devices and their delivery methods. We also summarize the reactive species generated by CAP and their detection methods. Moreover, the applications of CAP against chronic diseases and their underlying mechanisms are examined, especially how CAP can be used as a precise and minimally invasive treatment in the long-term management of chronic conditions. These insights presented in this review may facilitate the development of more effective CAP-based therapies and support the integration of CAP into clinical practice, which has the potential to advance the clinical translation of CAP technology and achieve long-term, safe, effective, and accessible care for chronic conditions.

2 Overview of CAP

2.1 History of plasma development

In medicine, "plasma" denotes the acellular fluid of blood. By analogy, the same term was adopted in physics after Sir William Crookes first observed it as "radiant matter" in 1879, and Irving Langmuir later coined it in 1927 to describe an ionized fluid transporting electrons and ions [26–28]. Low-pressure cold plasma emerged in the late 1960s for surface decontamination but was unsuitable for tissue use due to cost and operational demands. The biomedical potential of CAP was not realized until the mid-1990s, when Laroussi demonstrated an atmospheric pressure plasma for microbial inactivation. In 2013, the first medical plasma devices reached the European market as Class IIa devices and have since shown clinical efficacy in oncology, wound care, and dermatology [29–31].

Beyond biomedicine, plasma has also advanced agriculture, catalysis, food processing, material synthesis, and environmental remediation [16, 27, 32–38]. Unlike conventional high-temperature plasma, CAP operates at room temperature and atmospheric pressure. It is a partially ionized and highly reactive gas, which includes neutral atoms and molecules, electrons, ions, reactive oxygen and nitrogen species (RONS), along with electric fields and ultraviolet (UV) photons generated during discharge. The therapeutic efficacy of CAP arises from the synergistic action of these components, particularly RONS, while its non-thermal nature ensures biocompatibility [39]. With demonstrated efficacy against cancer, chronic wounds, and other chronic diseases, CAP represents a promising therapeutic modality (Fig. 1).

2.2 CAP-generated reactive species for delivery

CAP is mainly formed by applying electrical energy to gas (argon, helium, oxygen, nitrogen, ambient air, or mixtures thereof) at atmospheric pressure [18]. When an electric field is applied to the gas, free electrons are accelerated and gain high kinetic energy, becoming "hot" electrons. These energetic electrons collide with neutral gas particles, triggering Townsend avalanches that lead to ionization events and the release of additional electrons. Despite this, the bulk gas remains mostly neutral, with only a small fraction ionized at any given moment. Due to their greater mass, gas atoms and ions accelerate inefficiently in the electric field, and the inefficient transfer of kinetic energy from electrons to these heavier particles keeps them in a low-energy state, thereby maintaining a low plasma temperature and minimizing thermal effects on treated materials [16, 40]. The excited and ionized species subsequently interact with adjacent media, such as air, liquids, and surfaces, generating reactive species with biological potential. In addition, the excitation process also emits electromagnetic radiation, including UV rays, visible light, infrared radiation (IR/heat), and electric fields, all of which contribute to the biological effects [41].

The composition and quantity of reactive species vary significantly across different gas environments, with the feed gas type being a key determinant of RONS production [42]. Oxygen-containing plasmas generate ·OH, O_3, and H_2O_2, whereas nitrogen-containing plasmas produce N, N_2^+, and N^+ [38]. In addition, other operational parameters—including gas flow rate, driving current, gap distance, and ambient humidity—also influence reactive species yields [43].

Beyond gas-phase reactions, plasma interactions with air generate ·OH and nitric oxide (NO), while plasma-liquid interactions yield longer-lived species such as nitrites (NO_2^-), nitrates (NO_3^-), and H_2O_2 [44]. These species can diffuse into liquids and undergo secondary reactions to form plasma-activated liquids (PAL) or plasma-activated hydrogels (PAH), which contain both short-lived ($ONOO^-$, ·OH, NO·, O_2^-, etc.) and long-lived

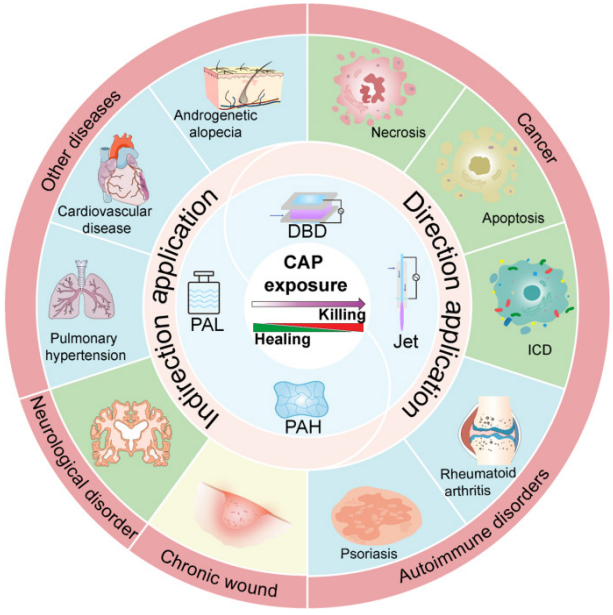

Figure 1 Delivery approaches and clinical application landscape of CAP in chronic disease therapy.

(O_3, H_2O_2, NO_2^-, etc.) RONS considered critical for therapeutic efficacy [45–50].

In addition to RONS, CAP emits UV photons at discrete wavelengths corresponding to electron transitions in atomic or molecular shells [32]. These UV photons can rapidly generate ·OH in water to millimeter depths via photolysis, contributing to the overall biological activity alongside directly delivered RONS [51–53].

2.3 Importance of CAP in biomedical applications

CAP exhibits multiple remarkable biological effects that are primarily dependent on RONS. RONS serve as multifunctional signaling molecules, participating in normal biological processes and potentially initiating harmful responses at higher levels [54]. Thus, by regulating the concentration of RONS generated by CAP, it is possible to either elicit beneficial cellular physiological signals or induce cell death. In terms of sterilization, CAP exhibits extensive microbial inactivation, encompassing multidrug-resistant strains, while exerting distinctly different influences on cells based on various treatment intensities [41]. Specifically, in cancer cells, the action mechanism of CAP is linked to the elevation of intracellular reactive oxygen species (ROS). This is due to the fact that RONS generated by CAP possess potent oxidative properties, capable of peroxidizing unsaturated fatty acids, affecting lipids, proteins and DNA, and triggering redox-related signaling pathways within cells. These pathways induce a diverse array of cellular effects, including cell death, cell detachment, alterations in cellular morphology, changes in metabolic pathways, modified cell migration patterns, alterations in cell motility, and either enhancement or suppression of cell proliferation [55]. In conclusion, low-intensity CAP promotes cell proliferation and tissue regeneration, whereas high-intensity CAP induces cell inactivation through apoptosis [56].

3 Cold atmospheric plasma: Technology and mechanisms

To utilize CAP as a safe and reliable therapeutic modality, in-depth understanding of the mechanisms of plasma generation and their associated effects is crucial for developing reliable plasma sources. Additionally, comprehending the needs for plasma in disease treatment and the bottlenecks encountered in its delivery is essential for clinical applications. The various CAP application methods for chronic diseases, as discussed in this review, are summarized in Table 1. Furthermore, investigating the composition and concentration of plasma-generated reactive species and their impacts on biological systems—including microorganisms, cells, and tissues—necessitates continued advancements in plasma diagnostics. Accordingly, this review aims to systematically synthesize and compare the currently reported plasma generation devices and delivery strategies that are safe, stable, and controllable, thereby providing practical technical guidance for the diverse therapeutic needs in chronic disease management.

3.1 Cold plasma technology and delivery methods

3.1.1 Types of cold plasma technology

Medical CAP sources have various design and engineering concepts, such as plasma jets, dielectric barrier discharges (DBD), microwaves, electron cyclotron resonances, and capacitively coupled devices [32]. This section will focus on both plasma jets and DBD devices, which are major sources of CAP [89]. These devices can directly influence living organisms or activate mediators for indirect disease treatment. They can also generate a chemically rich environment for human tissues in a beneficial and nondestructive manner.

DBD is a self-sustaining electrical discharge in electrode configurations containing an insulating material in the discharge path [90]. The electrodes can be covered with a dielectric layer individually or collectively, or the dielectric material can be positioned in the interelectrode space, thereby providing a unique method for establishing a nonequilibrium atmospheric pressure discharge. Additionally, DBD devices require alternating or pulsed high voltage for capacitive coupling. Owing to the presence of an insulating dielectric layer in its discharge structure, DBD can effectively inhibit the occurrence of local sparks or arcs. Its fundamental discharge mode manifests as filamentary discharge, which subsequently facilitates the production of large-area, high-energy-density, low-temperature non-equilibrium plasmas.

Non-equilibrium atmospheric pressure plasma jets (N-APPJ), generate plasma in open space rather than in a confined discharge gap, consisting of a gas nozzle equipped with one or two electrodes [41]. Compared with DBD devices, N-APPJ generate high-density plasma plumes in smaller volumes by ionizing a supplied feed gas at the electrode. Subsequently, this plasma plume is then guided and transported outside the device. Their long plume length of several centimeters makes it possible to access the inner space of three-dimensional structures [91, 92]. This enables more precise and flexible treatment of localized areas, particularly in applications that demand high spatial and temporal control of plasma characteristics. One of the main factors affecting the performance of N-APPJ is the composition of the feed gas [93]. Noble gas plasma jets, though easy to generate, are less reactive than air plasma jets. For biomedical applications, a small percentage of reactive gases such as O_2 can be added to the noble gas, which serves as the carrier gas to produce the more reactive plasma [94–96]. These characteristics make N-APPJ particularly attractive for biological and medical applications.

3.1.2 Cold plasma delivery methods

The two main types of CAP applications in research and

Table 1 A summary table of chronic disease-related CAP delivery applications

Delivery	Application method	Type of disease treated	Performance	Ref.
Direct CAP delivery	Injection/surgery	Rheumatoid arthritis	- Induce RA-FLS apoptosis, alleviate RA symptoms, enhance antioxidant capacity	[57, 58]
	Wearable plasma devices	Wounds and bacterial infections	- Promote wound healing, exhibit antibacterial activity, cause no cytotoxicity or skin irritation	[59]
		Psoriasis	- Open Calcium channels in keratinocytes, restore differentiation, tight junctions, alleviate psoriasis	[60]
	Portable air-fed CAP device	Cancer	- Induce immunogenic death, boost T-cell response	[61]
	DBD	Neural differentiation	- Induce neural differentiation through cross-talk between the specific RONS cascade and Trk/Ras/ERK signaling pathway	[62]
	Plasma torch	Chronic wounds	- Decrease bacterial load of chronic wounds and promote healing	[63]
	Plasma jet		- No therapy-related serious adverse events - Promote diabetic wound healing, reduce inflammation, enhance re-epithelialization and collagen deposition - Downregulate IL-6, TNF-α, inducible nitric oxide synthase (iNOS), and SOD, while upregulating vascular endothelial growth factor (VEGF) and transforming growth factor-β (TGF-β) - Accelerate pressure ulcer healing via enhanced re-epithelialization, angiogenesis, and collagen deposition with reduced inflammation	[64–67]
Indirect CAP delivery	Plasma-activated culture medium	Cardiac ischemia/ reperfusion injury	- Protect mouse hearts against ischemia/reperfusion (I/R) injury by preventing super-sulfide catabolism	[68]
	Plasma-activated water	Cancer	- Apoptosis efficiency was dependent on the plasma exposure time and on the levels of ROS and RNS.	[69]
	Plasma-activated nebulized mist	Sterilization	- Inactivate different microorganisms including bacteria	[70]
	Plasma activated cryo-microneedles patches	Cancer	- Induce both ferroptosis and apoptosis to selectively kill tumor cells	[71]
		Psoriasis	- Trigger ROS-mediated apoptosis and mitochondrial damage, curbs keratinocyte growth	[72]
	Indirect PAH	Microbial disinfection	- Achieve long-term effective antibacterial	[47]
		Post-surgical treatment of cancer	- Eliminate residual tumor tissues post-surgery and inhibit recurrence without evident systemic toxicity	[73]
		Vitiligo	- Reduce immune cell infiltration, suppress inflammatory mediators, and activate a robust antioxidant stress response	[74]
		Cancer	- Release RONS within 30 min to kill osteosarcoma cells and elicit immunogenic signals, promoting DC-mediated phagocytosis	[75]
	Direct PAH	Vocal fold injury	- Improve the mechanical properties of hydrogel, activate fibroblasts, promote wound healing and reduce scar formation	[76]
		Cancer	- Have a sustained anticancer effect and prolong the storage duration of reactive species - Sustained ROS accumulation and release induce melanoma cell apoptosis	[77]
Device-assisted plasma delivery	Micron sized hollow tube	Cancer	- Suppress tumor growth and induce tumor cells apoptosis	[78]
	Hollow-structured microneedle patches	Cancer	- Boost CAP delivery, enhance immunity, and inhibit the tumor growth of both primary tumors and distant tumors combined with aPDL1	[79]
	Microneedle patch	Androgenetic alopecia	- Microneedle patches transiently microchannel the skin to deliver CAP-generated NO to hair follicles - CAP synergizes with microneedles and minoxidil to treat androgenetic alopecia	[80]
	Electrical plasma NO generation	Pulmonary hypertension	- Generate NO from air, scavenge pollutants, and deliver purified NO for pulmonary hypertension therapy	[81]
Combination of CAP delivery and other therapeutics	ICB	Cancer	- Impair the growth of distant tumors - Evoke both strong innate and adaptive, local and systemic anti-tumor immune responses - Inhibit both tumor growth and potential metastatic spread	[82, 83]
	Nanoadjuvants	Cancer	- Trigger both gelation and immunogenic tumor cell death - Remodel the tumor immune microenvironment	[84]
	Antimicrobials	Sterilization	- The on-demand trigger release of the drug using CAP	[85]
	Chemotherapy	Cancer	- Sensitize tumors to doxorubicin via ROS while sparing bone cells - Cisplatin/CAP synergistically kill tumor cells, sparing normal cells	[85, 86]
	Nanoparticles	Cancer	- Synergistically kill lung cancer cells by reducing viability and inducing apoptosis - Reduce the level of drug resistance in tumors	[87, 88]

biomedical trials are direct plasma treatments and indirect plasma treatments [97]. In addition, plasma can also be used in combination with other treatment methods (Fig. 2). Direct plasma treatments rely on short-lived species such as ·OH, $^1O_2$, O_2^-, NO·, $ONOO^-$, and atomic radicals, all of which are directly formed by the plasma. In contrast, the efficacy of the indirect plasma treatments relies on the generation of relatively long-lived reactive species, such as NO_x, H_2O_2, O_3, and additional secondary radicals produced in the liquid phase [98]. Direct plasma treatments are the most straightforward. The N-APPJ and DBD have been extensively researched and applied in the field of direct CAP therapy for diseases [66, 99–101]. The utilization of flexible electrodes for the fabrication of wearable plasma devices, such as wearable plasma gloves and patches, not only overcomes the limitations of treatment areas but also achieves better conformity to the human body, thereby making the application of direct plasma treatments more convenient and effective [102]. Kim et al. developed a wearable plasma pad kit utilizing a flexible polymer film capable of adhering to human skin. When coupled with a wearable power supply unit, this can be flexibly and conveniently utilized for beauty and skin treatment applications. It has been demonstrated to impact the activity of bacteria on the skin and promote wound healing [59].

CAP can also be used directly to resect tumor tissue. For example, Canady et al. treated liver cancer using Canady Helios Cold Plasma Scalpel to remove cancerous tissue without damaging the blood supply to the remaining liver [103]. The clinical data on plasma treatment for chronic wounds indicate that it is a process involving the synergistic action of multiple therapeutic mechanisms. Plasma treatment effectively reduces bacterial load, promotes angiogenesis, and enhances re-epithelialization. These effects significantly accelerate various stages of wound healing, demonstrating favorable therapeutic outcomes [40, 63].

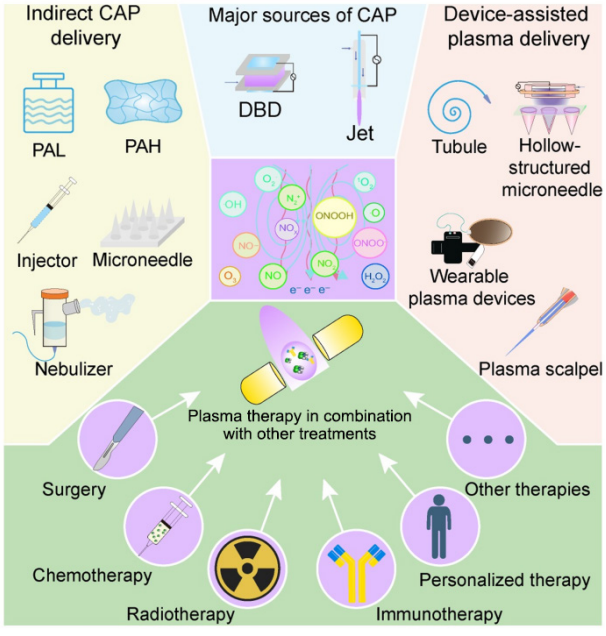

Figure 2 Overview of CAP composition, administration strategies, and combinatorial therapeutic regimens. The CAP environment consists of the CAP generating reactive species, electrons, other ions, radiation, waves, and physical forces. The main CAP generation devices are DBD and Jet. CAP can be applied directly via device-assisted delivery, such as tubes, hollow microneedles, and wearable devices, or indirectly via PAL, PAH, or nebulized liquid. Currently, CAP can be used alone or in combination with conventional chronic disease treatments, such as surgery, chemotherapy, and immunotherapy, demonstrating great potential for clinical application and translation.

3.1.3 *Biomedical device-assisted plasma delivery*

Most plasma systems are restricted by factors such as difficult-to-reach regions, treatment of internal organs, and the limited penetration depth of reactive species [104]. They are primarily used for treating easily accessible lesions and superficial tissues. To enable deeper tissues or internal treatment, biomedical devices are employed to deliver plasma, allowing reactive species to be transported away from the source, reach internal organs, access hard-to-reach areas, or enhance plasma penetration into deeper tissue layers. Using small tubes to deliver reactive species directly to the target with plasma devices is an effective solution for biomedical applications [105]. Mirpour et al. employed subcutaneous injection of a capillary tube to assist in plasma delivery to tumor sites, while inserting a capillary on the opposite side of the tumor to expel gases, thus achieving minimally invasive plasma-based tumor treatment and significantly inhibiting tumor growth [78]. Chen et al. pioneered a 70 μm diameter plasma jet that delivers plasma via an intracranial endoscopic tube to precisely treat glioblastoma tumors in the mouse brain. The tumor volume treated with the He μCAP decreased by nearly 50% compared with baseline levels [106]. In addition to this, using a flexible dielectric tube, the plasma jet bullets can be transmitted flexibly and precisely over long distances. This facilitates the potential use of plasma in endoscopic treatments [107, 108].

Microneedles serve as a novel drug delivery vehicle. They can mechanically break the stratum corneum, thereby enhancing the permeation of therapeutic agents into the dermis in a minimally invasive and painless manner [109]. Due to these properties, microneedles can serve as an auxiliary carrier for plasma delivery, offering an effective therapeutic approach for chronic diseases [110, 111]. Chen et al. designed a hollow-structured microneedle hMN patch in which each microneedle serves as a microchannel to deliver CAP through the skin into the tumor tissue (Fig. 3) [79]. This design represents a significant advancement in the field of plasma medicine, as it not only overcomes the limited tissue penetration of CAP but also enables the combined use of CAP with anti-programmed death-ligand 1 antibody (aPDL1) to further enhance anti-tumor immunity (Fig. 3(a)). Consequently, it achieves inhibition of both primary and distant tumor growth, prolonging the survival of tumor-bearing mice (Figs. 3(d) and 3(e)).

3.1.4 *Biomedical application modes of PAL*

Most previous studies focus on the direct application of CAP for disease treatment. However, when CAP technology is applied clinically for the treatment of localized focal lesions, stringent requirements about both the duration of treatment and the spatial arrangement of CAP application emerge, ultimately hindering the widespread clinical adoption of direct CAP therapy [77]. Although biomedical devices are employed to deliver plasma, partially overcoming spatial barriers, therapeutic efficacy is still limited. This is due to the difficulty in maintaining the activity of the plasma-generated reactive species over time, which poses a challenge for prolonged plasma application. A major advantage of plasma-activated media, including PAL, PAH, lies in their ability to store these reactive species for relatively extended periods [44].

Common PALs include plasma-activated water (PAW), plasma-activated saline solutions (PASS), and plasma-activated medium (PAM). PAW is an acidic solution produced from the interactions

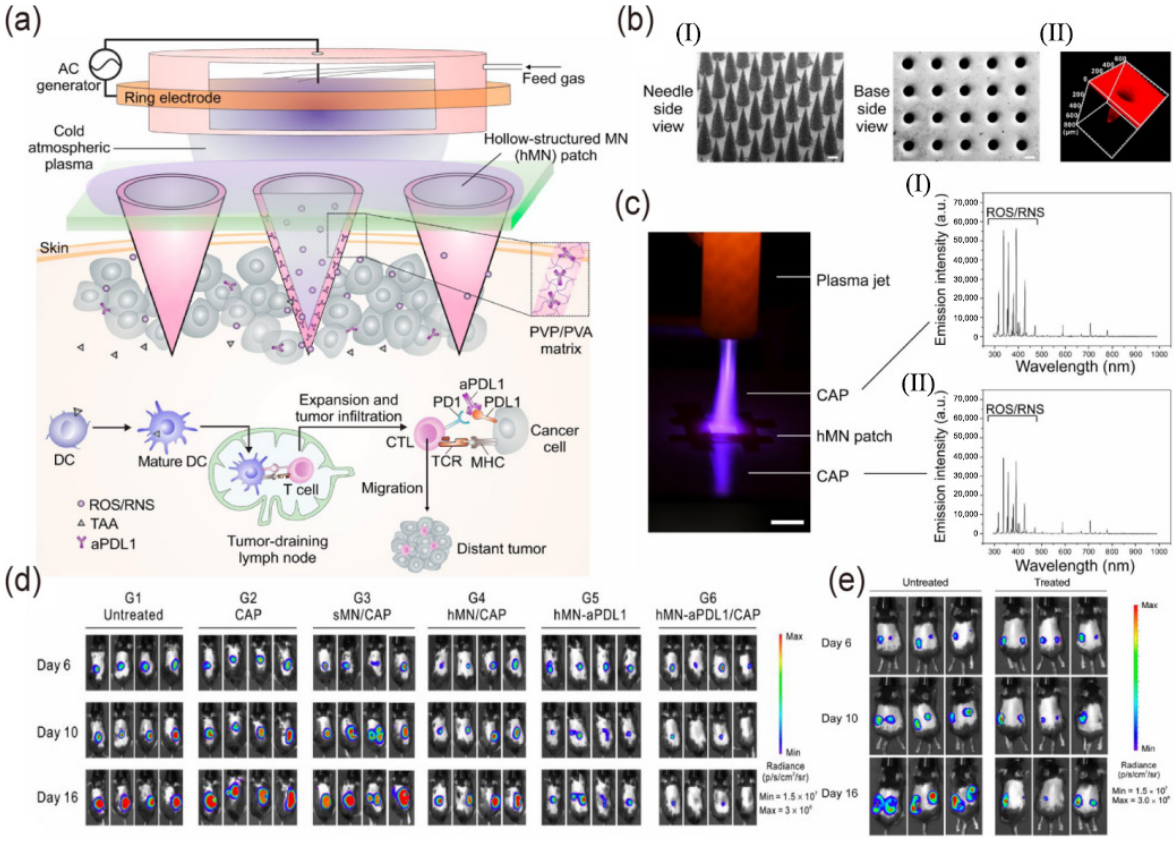

Figure 3 Illustration of the transdermal CAP-mediated ICB therapy. (a) Schematic illustration of the transdermal combination for CAP immunotherapy assisted by hMN patches loaded with aPDL1. (b) Representative images of the hMN patches: (I) representative SEM images of the needle side and base side of the hMN patches; (II) three-dimensional CLSM image of the hMN patch (loaded with rhodamine). (c) Penetration test of CAP through the hMN patches: (I) representative OES spectrum of CAP above the hMN patch; (II) representative OES spectrum of CAP penetrating through the hMN patch. (d) Bioluminescence of tumors in untreated mice and mice from various experimental groups. (e) CAP and hMN-aPDL1 inhibit distant tumor growth, as shown by the suppression of tumor bioluminescence in untreated and treated mice [79]. Reproduced with permission from Ref. [79], © Published under the PNAS license 2020.

between water and CAP by either direct plasma discharge in water or plasma discharge above the water surface. The biochemical activity of PAW is linked to the concentration of RONS in the water, particularly stable ROS and reactive nitrogen species (RNS) like H_2O_2, NO_3^-, NO_2^-, and the reactive species formed from them [112]. The synergistic effect of these RONS and the low pH plays a key role in the oxidative stress to bacterial cells and in the destruction of DNA within cells [113]. Importantly, liquid can help ROS penetrate spheroids and prolong the anticancer effects of plasma [114, 115]. Additionally, the RNS in PAL have been reported to contribute to synergistic antimicrobial effects and may also enhance cytotoxicity [116].

The preparation method of PASS is similar to that of PAW. In PASS, different reactive chlorine/oxy-chlorine species (RCS) are generated alongside ROS/RNS, all of which play critical roles in biomedical applications [117]. While retaining the antitumor activity of plasma, the generation of RCS enhances PASS sterilizing capacity. However, this also leads to a lower concentration of RONS compared to PAW. Besides the PAW and PASS mentioned above, PAM have demonstrated cytotoxic activities in various cancer therapies both *in vitro* and *in vivo*.

Currently, the main methods of using PAL for disease treatment include oral administration, direct irrigation of the lesion, and subcutaneous injection to penetrate the skin for the treatment of internal lesions [69]. To meet the clinical treatment and prevention needs of diseases, there is a need to develop more effective and specialized delivery methods for PAL. Wu et al. prepared air-discharge plasma-activated ice microneedles by rapid freezing of plasma-activated saline with higher bioactivity at −80 °C. These microneedles hold great potential for transdermal delivery of RONS, offering a promising approach for the treatment of psoriasis by overcoming the limitations of direct CAP application in clinical practice [72]. Similarly, Zhang et al. used a fast cryogenic micro-molding method to develop a novel RONS-loaded plasma-activated cryo-microneedles patch. This microneedle efficiently transports exogenous RONS across the skin with minimal invasiveness and completely dissolves after delivery. Through a complementary ferroptosis/apoptosis mechanism, the patch induces melanoma cell death, with no significant toxicity to normal cells [71].

Nebulization can serve as an alternative method for the delivery of PAL, offering broad potential for biomedical applications. Plasma-activated nebulized mist generated via spray reduces droplet size and increases the surface area-to-volume ratio, thereby enhancing the transfer of reactive species generated by plasma into water and the delivery of short-lived species [118]. Research indicates that plasma-activated nebulized mist efficiently inactivates airborne pathogens and eradicates microorganisms on treatment chamber surfaces and mouse skin without causing biological toxicity [70].

3.1.5 *Biomedical application modes of PAH*

PAL has emerged as an effective carrier of plasma-induced RONS,

which partly overcomes the spatial restrictions of plasma direct treatment. However, injection of a liquid into the body may lead to rapid diffusion due to extracellular fluids and blood flow, consequently diluting the RONS. This still limits its application and effectiveness in plasma-based disease therapy. Therefore, developing efficient vehicles for local confinement and delivery of RONS to the diseased site is a fundamental requirement [56]. Recently, numerous types of PAH have aroused wide concern among researchers for their stability and good biomedical effects, leading to further exploration in several biomedical fields.

Hydrogels are 3D hydrophilic polymer networks formed via physical entanglement, electrostatic interactions, or covalent cross-linking [119, 120]. With excellent biocompatibility, they have been widely explored for chronic disease treatment [121, 122]. They absorb large volumes of water and respond to pH, temperature, and ROS with changes in structure, swelling, permeability, and mechanical strength. These properties enable hydrogels to be activated by plasma and support the long-term storage of plasma-activated reactive species, which results in the formation of PAH [56, 123, 124]. PAH can be prepared by direct and indirect plasma activation of the gel materials (Fig. 4(a)). For direct PAH preparation, gel powders were mixed with deionized water to prepare hydrogels and subsequently treated with plasma. For indirect PAH, deionized water was first activated by plasma, then mixed with hydrogel powders [47, 125].

It is worth noting that the material of the hydrogel has an important influence on the biological activity of PAH. PAH stores reactive species generated by the plasma over extended periods and regulates their localized, sustained release. This process effectively kills osteosarcoma cells and induces the release of immunogenic signals, thereby enhancing the phagocytic uptake of these signals by human monocyte-derived dendritic cells (DCs), enhancing the therapeutic efficacy of plasma in disease treatment [75]. This study aims to demonstrate the immunogenicity of PAH therapy, providing a theoretical basis for exploring combination treatment strategies. Hydrogel materials have been shown to improve the therapeutic effects of plasma. For example, trehalose, a natural disaccharide, can enhance CAP-induced immunogenic cell death (ICD) by elevating the phosphorylation of eIF2α [83]. PAHs can serve as vehicles for RONS delivery to treat tumors, and also incorporate medicines to enhance anti-tumor and kill pathogenic microorganisms [82]. Besides activating hydrogels, CAP can also be used to induce *in situ* gelation and to modify the hydrophilicity of the material (Fig. 4(b)) [126], enhancing the mechanical properties of hydrogels [76, 127], and triggering the on-demand release of drugs from hydrogels (Figs. 4(c) and 4(d)) [128, 129].

3.1.6 Guiding principles for CAP delivery mode selection

In CAP therapy, superficial lesions (e.g., skin wounds, psoriasis) are best managed with direct plasma, while deep-seated tissues favor indirect PAL/PAH delivery for sustained efficacy. Device-assisted approaches offer distinct advantages in specific clinical scenarios by combining the abundant reactive species characteristic of direct treatment with the tissue-penetrating capacity inherent to indirect methods. The core principle is that modality selection should be disease-driven rather than availability-driven-matching RONS profiles and spatiotemporal dynamics to clinical needs. This application-driven paradigm is essential for CAP standardization and clinical translation.

3.2 Dosimetry of CAP

The high complexity of reactions occurring in plasma-air and plasma-liquid interactions, along with the formation of stable and unstable intermediates and reaction products, as well as the structural similarities of several chemicals involved in these reactions, necessitates the use of highly sophisticated analytical methods [130]. Currently, the reactive species generated by CAP can be identified using various experimental and instrumental techniques [32]. The methods employed for characterizing gas-phase plasmas include optical emission spectroscopy, mass spectrometry, optical absorption spectroscopy, and microwave or heterodyne interference measurements. For plasma-activated liquids, electron paramagnetic resonance spectroscopy, ultraviolet-visible absorption spectroscopy, mass spectrometry, and direct chemical characterization techniques are utilized for their identification. This section discusses several primary characterization methods for plasma-activated species used in biomedicine [131, 132].

Because of its experimental simplicity, optical emission spectroscopy (OES) is a commonly used diagnostic method for all forms of CAP. Optical emission spectroscopy probes only the excited levels because it measures the radiation emitted by the excited atoms or molecules in the plasma, as it is entirely passive in its mechanism and can be used simultaneously with active experimentation and subject treatment. OES can determine important plasma parameters such as gas temperature, vibrational temperature, electron temperature, electron density, types of reactive species, and even the absolute concentrations of some species [133]. To detect the population density and energy state of active species in living cells and tissues with high spatial and temporal resolution, laser-induced fluorescence (LIF) can be used. LIF is perfect for measuring the concentrations of the reactive species in the ground states. Although LIF detection systems are complex, they have been used to study the density distribution and temporal behavior of oxygen reactive species (OH and O radicals) produced by helium plasma jets [134, 135]. Spectrophotometry can also be employed to characterize treated samples, such as Fourier transform infrared spectroscopy (FTIR), and ultraviolet visible spectroscopy (UV–vis) [136, 137].

If spectral radiation is weak or absent, optical absorption spectroscopy (OAS) is a better alternative to OES. In OAS, particles inside the plasma are regarded as absorbers for the electromagnetic waves (transmitted through or reflected at the plasma boundary) to estimate the concentration of reactive species in the plasma [138]. Molecular beam mass spectrometry (MBMS) is another important plasma diagnostic technique. It provides information on the absolute density of ROS [139]. Electron spin resonance (ESR) spectroscopy, also called electron paramagnetic resonance (EPR) spectroscopy, is also used in the identification of short-lived radical reactive species in water-based media (such as $O_2^{\cdot-}$, ·OH, $ONOO^{-}$, and NO·) [140]. Due to the transient nature of these radicals, direct *in-situ* diagnosis is often impractical, necessitating the application of a technique known as spin trapping to treat the liquid samples [141]. The underlying principle of ESR in detecting these short-lived radicals involves their reaction with spin traps within the medium, resulting in the formation of long-lived spin-trapped adducts, which can subsequently be characterized using ESR spectroscopy. Importantly, since ESR measurement of these short-lived species is indirectly achieved through the use of spin-trapping,

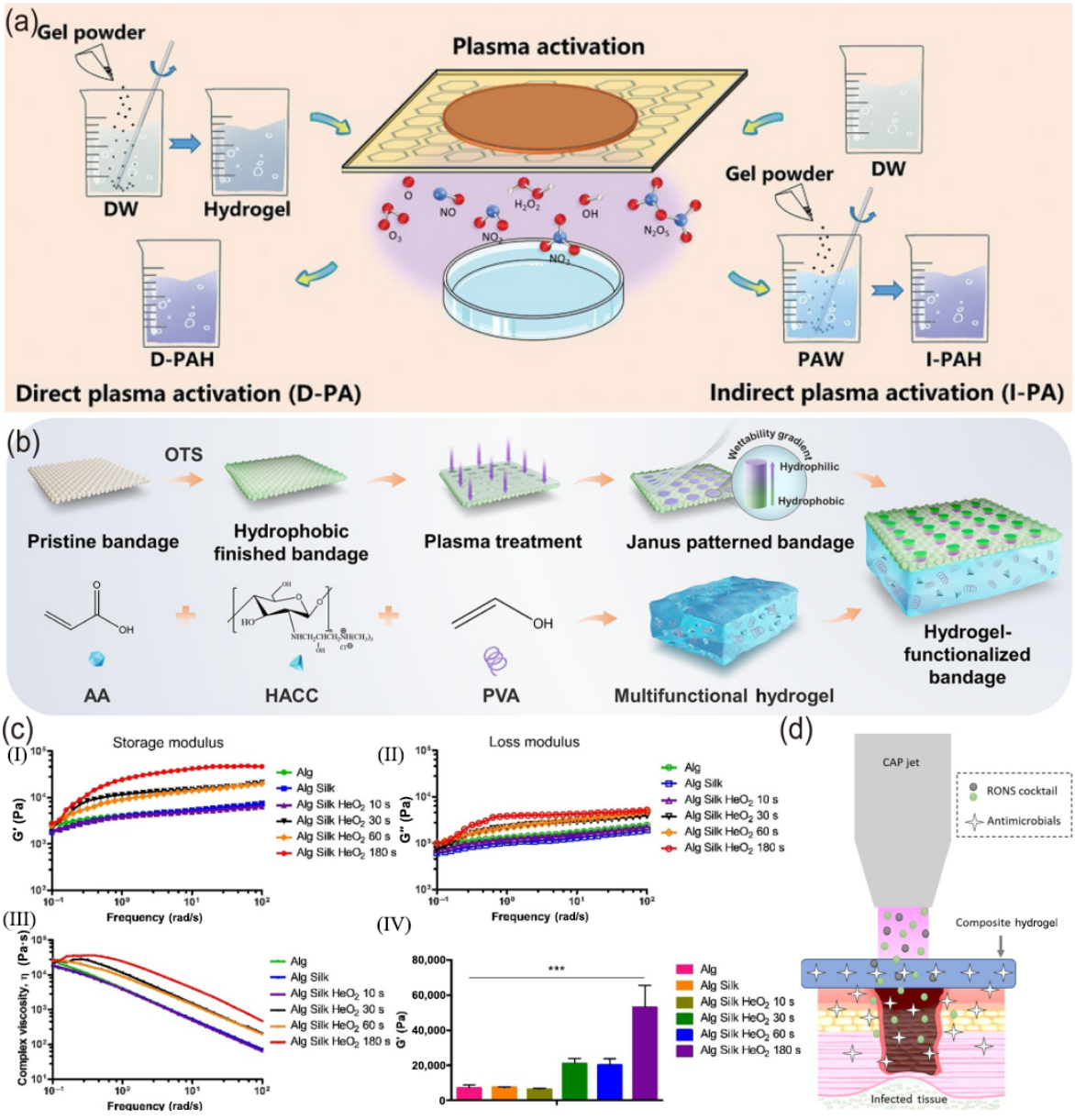

Figure 4 Biomedical application modes of PAH. (a) Preparation methods and properties testing of PAH: Direct plasma activation and indirect plasma activation of hydrogels [47]. Reproduced with permission from Ref. [47], © Chen, J. et al. Advanced Science published by Wiley-VCH GmbH 2023. (b) Schematic illustration of the preparation of a Janus wettability hydrogel functionalized bandage using plasma [126]. Reproduced with permission from Ref. [126], © American Chemical Society 2024. (c) Rheological properties of hydrogels as a function of LTP treatment time [76]. Reproduced with permission from Ref. [76], © Kim, S. et al. Published by Elsevier Ltd. 2022. (d) Illustration of the concept of CAP-activated composite hydrogel therapy for an infected tissue [128]. Reproduced with permission from Ref. [128], © Gaur, N. et al. Published by American Chemical Society 2023.

the specificity of the traps employed has a direct impact on the ESR results obtained [142].

In addition to the above methods, scattering techniques, such as Thomson, Rayleigh, and Raman scattering can be utilized [143]. Besides, methods such as the fluorescent probe method, chemiluminescence method, electrochemical method, and colorimetry are also widely used for detecting RONS [77, 144, 145]. To better simulate the *in vivo* situation and incorporate the influence of environmental components in living tissue, more complex model liquids must be investigated, which will also make the analytical challenges more complex. Molecular dynamics simulations will become increasingly essential for elucidating the mechanisms of plasma-material interactions, mapping the intricate reaction cascade from the plasma/gas phase into the liquid environment, and ultimately assessing how this entire chain of events influences cellular and tissue responses [41, 146–149].

3.3 Mechanisms of CAP action on biological systems

The biological efficacy of CAP is mostly attributed to chemical components (e.g., RONS), while physical components (e.g., electromagnetic fields and UV radiation) probably have synergistic effects [30, 150]. Although the precise molecular mechanisms by which CAP elicits biological phenomena remain to be fully elucidated, a wealth of experimental evidence has confirmed the functional roles of CAP-generated RONS in anticancer activity, antimicrobial activity, modulating cell signaling, immunomo-

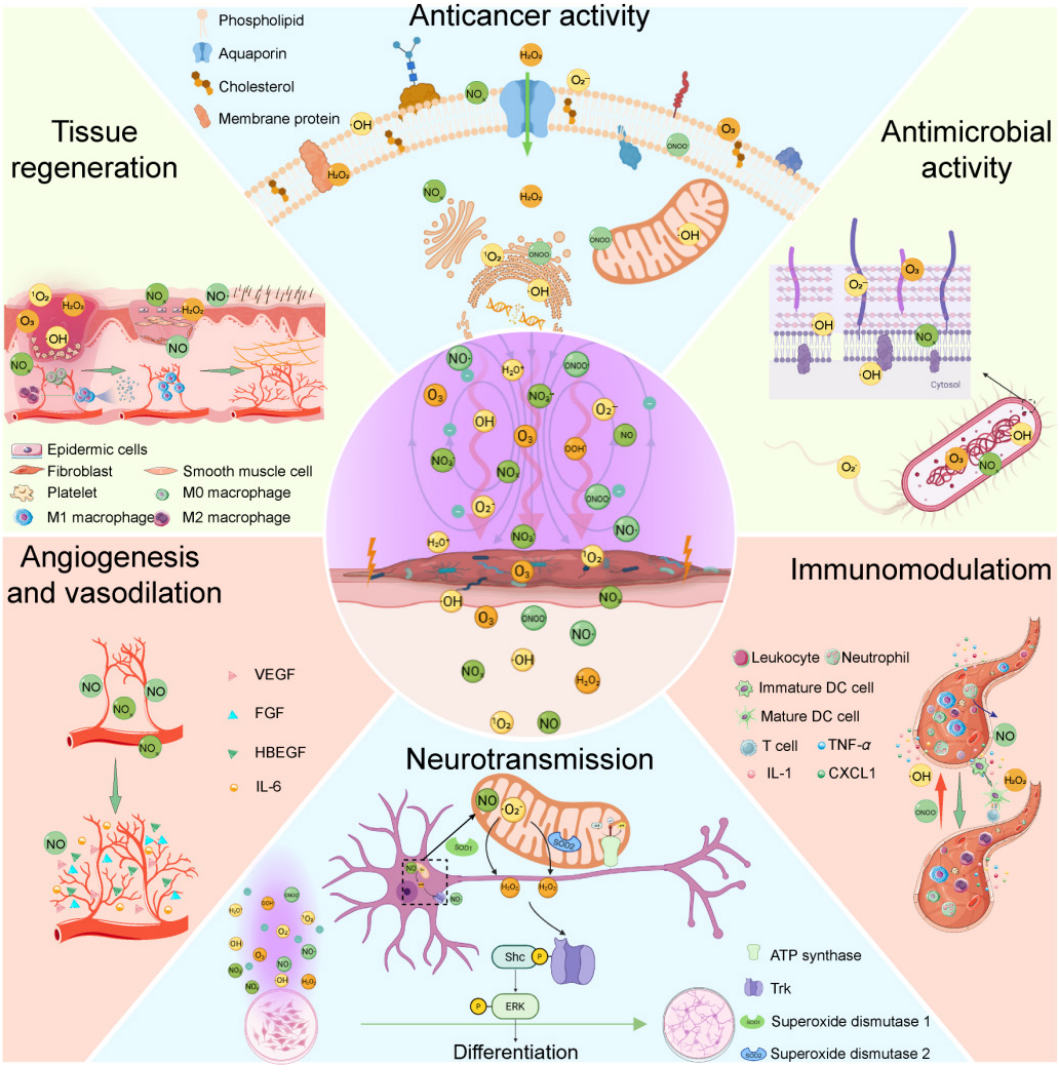

Figure 5 Schematic illustration of the main representative biological effects of CAP. The biological effects of CAP are primarily attributed to its reactive species, while its physical components (such as electromagnetic fields and UV radiation) may exert synergistic effects. Extensive experimental evidence has confirmed that the ROS and RNS generated by CAP play important roles in anticancer activity, antibacterial activity, immune regulation, and related biological processes. Moreover, the composition and concentration of RONS in CAP are key factors in determining its functions. Partially created with BioRender.

dulation, and related biological processes (Fig. 5). These observations have given rise to several mechanistic hypotheses.

The concentrations of both ROS and RNS in CAP determine their function, either physiologically beneficial regulation or pathological harmfulness. The molecular dynamics simulations reveal that CAP can leverage the differences in molecular expression between cancer cells and healthy cells, coupled with the combined effect of its electric field, to achieve efficient penetration and specific oxidative damage against tumor cells by RONS [151].

3.3.1 *Main short-lived species of CAP*

Common short-lived species include ·OH, O_2^-, $^1O_2$, NO·, and $ONOO^-$, which are crucial for bacteria inactivation than long-lived species [152].

·OH is often considered the main driver of biological effects, resulting from CAP when immediately exposed to the subject, owing to its status as a short-lived species of plasma-generated ROS. It is mainly formed within the plasma devices and possesses strong antimicrobial action, because of its high oxidative activity that results from the unpaired electron. It can efficiently bind to cellular materials by hydrogen bonding, participates in the destruction of cell membranes, and interferes with DNA and other intracellular components [151]. O_2^- is also short-lived in aqueous solutions and has limited ability to bypass cell membranes, leading to most oxidation driven by O_2^- occurring at the cell surface [153]. $^1O_2$ is a crucial oxidant in cells and tissues, generated through gaseous interactions within the plasma jet. $^1O_2$, with its relatively longer life span, can generate secondary singlet oxygen through an autoamplificatory process within cells, and exhibits stronger toxicity compared to ·OH [141]. Furthermore, $^1O_2$ can interact with specific redox biology-related enzyme compositions on the surface of tumor cells, and activate apoptosis signal receptors, thereby initiating intense and sustained biochemical and cellular reactions that specifically kill tumors [154].

NO· is a mediator in the regulation of vascular tone, neurotransmission, and immunity, among other metabolic and cell signaling effects. In several disease states, increased $O_2^{\cdot-}$ production, rapidly inactivates NO· through a diffusion-controlled radical termination reaction, forming the potent and short-lived oxidant $ONOO^-$ [155, 156]. $ONOO^-$ is a reactive, short-lived oxidant and

nucleophile that is important in various aspects of health and disease progression, including signal transduction and as a cytotoxic effector against invading pathogens [145, 155, 157]. At physiological pH, $ONOO^-$ can cross cell membranes and induce cell death through oxidation and nitration reactions, since excessive $ONOO^-$ levels can damage essential cellular components and organelles [158]. Accumulated cell damage ultimately results in apoptosis and necrosis [144].

3.3.2 *Main long-lived species of CAP*

The representative stable species are ozone (O_3), H_2O_2, NO, nitrogen oxides (NO_x), and other reactive components [159]. With half-lives ranging from seconds to days, these species are essential in indirect plasma therapy.

O_3, known for its antibacterial activity, oxidizes cell walls and membranes, enhancing permeability and causing cell rupture. It specifically targets thiol groups in microbial enzymes, disrupts glycosidic bonds in polysaccharides, affects nitrogen-containing amino acids and peptides, and interacts with bacterial nucleotides to release carbohydrates and phosphate ions, all contributing to its antibacterial efficacy [160, 161]. H_2O_2 is a key component in CAP that contributes to wound healing and exhibits antimicrobial and anticancer properties. Its formation is primarily driven by water vapor in the feed gas, allowing it to diffuse into liquid samples [162].

NO is water-soluble and can pass freely across cell membranes. It has a central role in signaling pathways involved in numerous physiological processes [163]. Providing NO from an external source is beneficial in promoting cellular functions and the treatment of different pathological conditions. The physiological role of NO is concentration-dependent. At extremely low concentrations, NO functions as an essential effector and signal transduction molecule in various cellular processes, such as post-translational modifications of proteins, regulation of gene transcription, mRNA translation, reproduction, apoptosis, neurotransmission, immune responses, and vasodilation [164]. In contrast, at elevated concentrations, NO could trigger oxidative stress and induce cytotoxicity, attributable to its capacity of altering cellular properties and targets [165]. NO_x exhibits multiple biological activities, and when dissolved in water, it forms two aqueous oxidizing acids (nitrous and nitric acids) and their conjugates (NO_2^- and NO_3^-) [166]. NO_x offers significant antimicrobial protection and has been shown to increase blood flow and mucus production in the gastrointestinal mucosa. Additionally, NO_x can synergize with H_2O_2 to enhance toxicity towards tumor cells [167]. Moreover, NO_2^-, as a component of NO_x, releases NO and other RNS in acidic and hypoxic environments, serving as a storage reservoir for NO in the body [168].

3.3.3 *Supporting role of other CAP components in therapy*

Apart from the pronounced biological effects of RONS generated by CAP on biological tissues and cells, other components of CAP also play auxiliary roles in therapeutic processes. For instance, CAP not only contains highly motile electrons but also exhibits conductivity, enabling it to convey electrical current to cells and tissues, thereby eliciting potential biological consequences and synergistic treatment effects [92, 169, 170]. It also exhibits large enough electric fields that may allow pharmaceutical molecules to enter the cells through cellular electroporation [100, 171, 172]. Additionally, UV photons assist RONS in deeply penetrating tissues and enhancing sterilization. Another possible explanation is the "bystander effect", where effects of plasma deep below the surface may be attributed to signaling between cells. This suggests that plasma-treated surface cells can transmit chemical signals to deeper layers, eliciting comparable responses (e.g., apoptosis). While this phenomenon shares similarities with quorum sensing and relies on intercellular communication, its exact mechanisms remain unclear and controversial [173]. These components collectively play a supportive role in the therapeutic process [43].

Despite being generally considered safe, not all RONS generated by CAP are beneficial in every circumstance. For example, concerns have been raised about the potential genotoxicity of CAP due to the presence of highly reactive ·OH [174]. Thus, elucidating the mechanism of action, exploring application scenarios, determining the dosage of active components, and optimizing the modes of use of CAP are essential tasks for researchers. These aspects are also focal points of this review.

4 CAP in chronic disease therapy: Current research and applications

4.1 CAP in cancer

In the past century, advancements in surgery, chemotherapy, and radiotherapy have demonstrated their capacity to effectively treat cancer, both as individual modalities and in combination [175]. These advancements have redefined cancer from a rapidly fatal disease to a manageable chronic condition, thereby altering its classification [176]. However, resistance has emerged to all currently available treatments, encompassing polychemotherapy, radiotherapy, immunotherapy, and molecular targeted therapy [167, 177]. CAP is emerging as a promising treatment in oncology. It is capable of inducing selective cytotoxicity in cancer cells *in vitro* and reducing tumor volume *in vivo* [178]. When integrated with other therapeutic modalities, CAP enhances the efficacy and selectivity of treatments against therapy-resistant cancers [140, 179–181]. To date, no significant resistance of cancer cells to plasma therapy has been reported [182, 183].

Recent studies have confirmed that CAP monotherapy exhibits significant cytotoxic effects against various malignant tumors [184]. CAP has been shown to effectively reduce the volume of solid tumors, prevent post-resection recurrence, and have minimal adverse effects on normal cells [185, 186]. Although the precise mechanisms by which CAP induces tumor cell death remain unclear, several studies have begun to elucidate potential pathways. This chapter will synthesize current research findings to systematically explore CAP's impact on tumor cell DNA, gene expression, cellular organelles, and modes of cell death when used in isolation. This comprehensive understanding will provide a foundation for the subsequent chapter, which will discuss CAP's synergistic effects when integrated with other therapeutic modalities.

Direct and indirect CAP therapies are primarily believed to induce cancer cell death through oxidative stress mechanisms. Cancer cells typically exhibit elevated levels of RONS and a more fragile antioxidant system compared to normal cells, rendering them more susceptible to redox stress and closer to the threshold of cell death [187]. Additionally, some researchers suggest that the physiological differences between cancerous and normal cells allow CAP-generated RONS to selectively trigger redox imbalances in

tumor cells. These mechanisms are not mutually exclusive but can act synergistically, enabling CAP to induce tumor cell death through various pathways, including apoptosis, autophagy, pyroptosis, ferroptosis, and necrosis [188]. The primary modes of tumor cell death induced by CAP are programmed cell death and impairment of cancer cell survival mechanisms [140, 189].

4.1.1 Selective cytotoxicity driven by differential membrane composition

The cell membrane serves as the natural interface between ROS generated by plasmas and the subsequent induction of distinct biological responses [31]. Variations in membrane composition between tumor and normal cells may influence the selectivity and efficacy of CAP treatment. Oxidation of all phospholipids on the plasma membrane can induce pore formation, which permits RONS to enter the cell and cause oxidative damage to intracellular macromolecules, such as DNA and proteins. However, increasing the cholesterol content in the lipid bilayer enhances membrane orderliness. When a certain threshold is reached, cholesterol can prevent pore formation, thereby protecting normal cells from RONS-induced damage. Cancer cells, due to their aberrant cholesterol concentration and distribution on cell membranes, are more susceptible to lipid peroxidation, which facilitates the penetration of RONS into the cells, inducing oxidative stress and apoptosis. Consequently, CAP therapy can selectively target and kill cancer cells without damaging healthy cells [182, 190].

The overexpression of specific aquaporin subtypes on the cell surface of cancer cells represents another pivotal mechanism underlying the selective eradication of cancer cells by CAP. This aquaporin promotes the passive diffusion of H_2O_2 across the cell membrane, thereby augmenting the intracellular concentrations of ROS. Consequently, elevated ROS levels enhance oxidative stress, ultimately inducing cancer cell death [191–193]. Furthermore, the function of specific aquaporin subtypes in modulating redox signaling via H_2O_2 transport across biological barriers is particularly intriguing, with significant implications for cellular motility and tissue repair [194]. Wang et al. investigated the effects of CAP on various types of head and neck cancer cells and elucidated the underlying mechanisms [140]. These include the E-cadherin/N-cadherin switch of epithelial-mesenchymal transition, apoptotic pathway, oxidation-reduction pathway of glutathione, glycolytic pathway, and PI3K/Akt/mTOR/HIF pathway. Leveraging bioinformatics analysis, multiple molecular pathways through which CAP exerts its anticancer effects have been elucidated. These pathways modulate key cancer processes, including proliferation, migration, invasion, epithelial-mesenchymal transition, oxidative defense, programmed cell death, and metabolic reprogramming.

4.1.2 Cell cycle-dependent sensitivity

Another explanation for the preferential cytotoxicity of CAP in tumor cells is based on the differences in cell cycle dynamics between normal and tumor cells [182]. Tumor cells, which are more frequently in the cell cycle, particularly in the S-phase, the DNA-replication phase, are targeted by plasma treatment. CAP induces apoptosis in S-phase cells by disrupting DNA replication and in M-phase cells by impeding sister chromosome segregation [195]. Additionally, the elevated RONS levels in tumor cells are exacerbated by CAP-generated species, potentially overwhelming the system and shifting the RONS effect from tumor-promotion to tumor-suppression [179]. This accounts for the heightened sensitivity of cancer cells to plasma treatment. Therefore, combining CAP with anticancer agents that target cell cycle "sensitive phases" may augment the therapeutic potential of CAP technology.

4.1.3 Induction of programmed cell death pathways

Specifically, CAP can induce tumor cell apoptosis through various pathways, contingent upon cellular characteristics, stimulus intensity, or the modulation of other signaling pathways. For instance, CAP-generated extracellular ROS activates tumor necrosis factor (TNF) signaling and TNF-dependent intracellular ROS generation. The elevated intracellular ROS levels, in turn, trigger caspase 3/7 activation via the consecutive engagement of apoptosis signal-regulating kinase 1 (ASK1) and the downstream p38 MAPK/JNK pathways, particularly the TNF-ASK1 pathway, thus selectively inducing apoptosis in human melanoma cells [196]. Furthermore, as a modality of indirect plasma therapy, PAM induces lung cancer cell death through a caspase-independent apoptotic pathway by disrupting the mitochondrial-nuclear network of cancer cells [197]. Other studies have also shown that PAM, unlike tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), effectively kills TRAIL-resistant cancer cells by triggering a calcium-dependent, caspase-independent cell death pathway, along with disruptions in mitochondrial dynamics and calcium homeostasis [198]. Similarly, Ahn et al. used a micro-plasma jet nozzle array to generate ROS, which depolarizes the mitochondrial membrane potential, induces mitochondrial dysfunction, and activates oxidative stress, ATP depletion, and mitochondrial autophagy, ultimately leading to apoptosis in HeLa cells [199]. Another study suggests that plasma exerts an apoptotic effect on B16 cells through the activation of the p53/PIGs/caspase signaling pathway [200]. Collectively, these findings highlight the therapeutic promise of plasma in targeting mitochondrial dysfunction and inducing apoptosis via diverse molecular mechanisms.

Beyond apoptosis, CAP can also trigger cell death via alternative pathways. Yang et al. demonstrated that CAP induces pyroptosis in a dose-dependent manner in tumor cell lines with high Gasdermin E expression. This pyroptotic process is triggered by the mitochondrial pathway (JNK/cytochrome *c*/caspase-9/caspase-3) and Gasdermin E cleavage. Additionally, ROS generated during CAP exposure initiates this pyroptotic signaling cascade [201]. Surprisingly, even at low doses, CAP still exhibits inhibitory effects on various tumor cells through mitotic catastrophe. This is due to the disruptive effects of low-dose CAP on microtubule dynamics and centrosome maturation, which induce mitotic catastrophe. Additionally, CAP-derived ROS cause severe damage and dysfunction to mitochondrial structures, a key factor in inducing mitotic defects [202].

4.1.4 Multifaceted biological effects of CAP beyond direct tumor cytotoxicity

In addition to the aforementioned benefits, plasma therapy for tumors also offers other advantages. Metelmann et al. used kINPen MED, a non-thermal plasma, to directly treat advanced head and neck carcinoma ulcerations and patients in the final stages of disease [203]. Clinical trials have shown that kINPen MED plasma-based cancer treatment significantly alleviates tumor growth, promotes the healing of oral and extraoral ulcerative lesions in patients with squamous cell carcinoma, and reduces microbial load along with associated odors. Similar to its effects in the treatment of chronic leg ulcers, plasma therapy also demonstrates a notable

analgesic effect in cancer treatment, thereby reducing the need for analgesic medications [204].

Multidrug resistance (MDR) and adverse effects remain significant challenges in cancer chemotherapy [205, 206]. MDR denotes the developed insensitivity of microorganisms and cancer cells towards chemotherapeutic drugs with diverse chemical configurations and distinct mechanisms of action [207, 208]. Extended contact with a singular chemotherapeutic agent can precipitate the emergence of resistance against multiple other structurally non-analogous agents, a phenomenon recognized as MDR. This phenomenon significantly obstructs the advancement of healthcare practices [209, 210]. Currently, clinically applicable methods to surmount MDR are constrained. Hence, there is an urgent imperative to identify novel approaches. Plasma treatment might serve as a viable alternative with significant potential. Research shows that CAP selectively eliminates tumor cells via multiple pathways and targets, which may explain both its low propensity to induce resistance and its ability to enhance therapeutic efficacy when combined with other treatments.

4.1.5 Synergistic cancer therapy: Combining CAP with small molecule chemotherapeutic agents

Extensive research has demonstrated the multifaceted anticancer effects when CAP is combined with various chemotherapeutic agents [23, 211], such as paclitaxel [212], doxorubicin [86], and cisplatin [85]. The combined treatment of PAM and doxorubicin specifically downregulates redox defenses and drug-resistance gene transcription. Increased doxorubicin uptake and intracellular ROS overload result from this approach. Moreover, PAM selectively amplifies doxorubicin's cytotoxic effect on targeted cancer cells without increasing toxicity in healthy cells, potentially offering an effective strategy to mitigate adverse side effects and reduce the required dose of doxorubicin [86]. The mode of combined treatment of CAP and drugs also affects the synergistic anticancer effect.

In certain instances, sequential drug treatment followed by plasma treatment offers more advantages, as plasma can restore sensitivity to doxorubicin in resistant cancer cells and augment its anti-tumor potential [95]. This is because CAP can suppress the growth of drug-resistant cancer cells and restore their sensitivity to chemotherapeutic agents by regulating the expression of multiple drug resistance-related genes. Specifically, this synergy arises from antioxidant phenotypic deficiencies in resistant cancer cells, marked by reduced antioxidant enzyme expression. This reduction augments PAM's ability to induce oxidative stress, effectively targeting doxorubicin-resistant cells. Additionally, PAM reverses resistance by downregulating drug resistance-related efflux pump MDR1 [213]. These findings suggest expanding CAP's application in treating drug-resistant tumors [214].

4.1.6 Synergistic cancer therapy: Combining CAP with surgery

For cancer therapy, to effectively inhibit cancer recurrence and reduce postoperative complications is very critical [215]. CAP can also be combined with surgical intervention to prevent postoperative tumor recurrence. For instance, Chen et al. utilized a portable air-fed cold atmospheric plasma (aCAP) for postsurgical cancer treatment (Fig. 6) [61]. The local aCAP therapy targets residual tumor cells in the surgical cavity, where the RONS generated by aCAP effectively induce cancer immunogenic cell death (ICD) *in situ* and locally release tumor-associated antigens (TAAs), thereby stimulating an effective antitumor immune response. Immature DCs phagocytose process TAAs into peptides during migration to tumor-draining lymph nodes, thereby facilitating antigen presentation by mature DCs to T cells, which subsequently drives the differentiation of cytotoxic T lymphocytes. T cell-mediated immune responses suppress tumor growth and recurrence. Utilizing 4T1 breast tumor and B16F10 melanoma models, studies demonstrate that aCAP treatment following incomplete tumor resection effectively induces a T cell-mediated antitumor immune response, which curbs local tumor regrowth and extends mouse survival (Figs. 6(b) and 6(c)).

The first clinical trial approved by the U.S. Food and Drug Administration (FDA) to use the Canady Helios Cold Plasma (CHCP) technology in combination with surgical treatment demonstrated the technology a safe and selective cancer treatment method. It can precisely eliminate residual tumor cells after surgery, thereby achieving effective control of local recurrence (Fig. 7(a)). This clinical trial further confirms the potential of CAP as a promising treatment for reducing the risk of tumor recurrence, laying a solid foundation for its future clinical application in cancer treatment [179, 216].

CAP therapy can directly eliminate residual neoplastic cells, thereby significantly reducing the risk of metastasis or tumor recurrence [217, 218]. Indirect plasma therapy for tumors can have a similar effect. PAL has demonstrated significant therapeutic efficacy in treating tumors prone to recurrence, such as bladder cancer and gastric cancer [219]. For instance, Yasuhiro's experiments in mice revealed that intraperitoneal injection of PAM significantly inhibits the peritoneal metastasis of gastric cancer (Fig. 7(b)(I)), reducing the formation of intraperitoneal tumor nodules by 60%. Additionally, it was observed that suspended gastric cancer cells exposed to PAM exhibited a marked reduction in adhesion capacity within a short contact time (Fig. 7(b)(II)). These findings suggest that intraoperative lavage with PAL as a strategy to prevent intraperitoneal dissemination holds considerable potential for clinical application [220].

Following surgical resection of tumors, plasma-activated thermosensitive biogel (PAPB) is injected into the surgical site. Since PAPB can prolong the storage duration of RONS, it results in a more sustained postoperative anticancer effect (Fig. 7(c)). This prolonged efficacy facilitates the long-term clearance of remnant tumor tissues after surgery, inhibits orthotopic recurrence, prolongs the survival period of mice, and demonstrates no significant systemic toxicity [73].

4.1.7 Synergistic cancer therapy: Immune activation by CAP

The regulatory effects of plasma on the tumor immune microenvironment (TIME) are very important for cancer fighting. Wang et al. achieved comparable outcomes in their experiments by combining CAP with cisplatin or immune checkpoint inhibitors (ICB), thereby significantly inhibiting tumor progression and extending the survival duration of mice [140]. Experimental findings from the combinatorial regimen of CAP and ICB indicate that CAP promotes the infiltration of $CD8^+$ and $CD4^+$ T cells into the tumor microenvironment, thus synergizing with aPDL1 immunotherapy. This mechanism enhances immune surveillance and activates cytotoxic T cells by disrupting cancer cell survival mechanisms and exposing tumor antigens. Besides, CAP modulates the tumor microenvironment (TME) to promote immune cell recruitment and infiltration. Further studies suggest that CAP exerts anti-tumor effects by inducing various forms of programmed cell death (such as apoptosis and autophagy) and weakening tumor

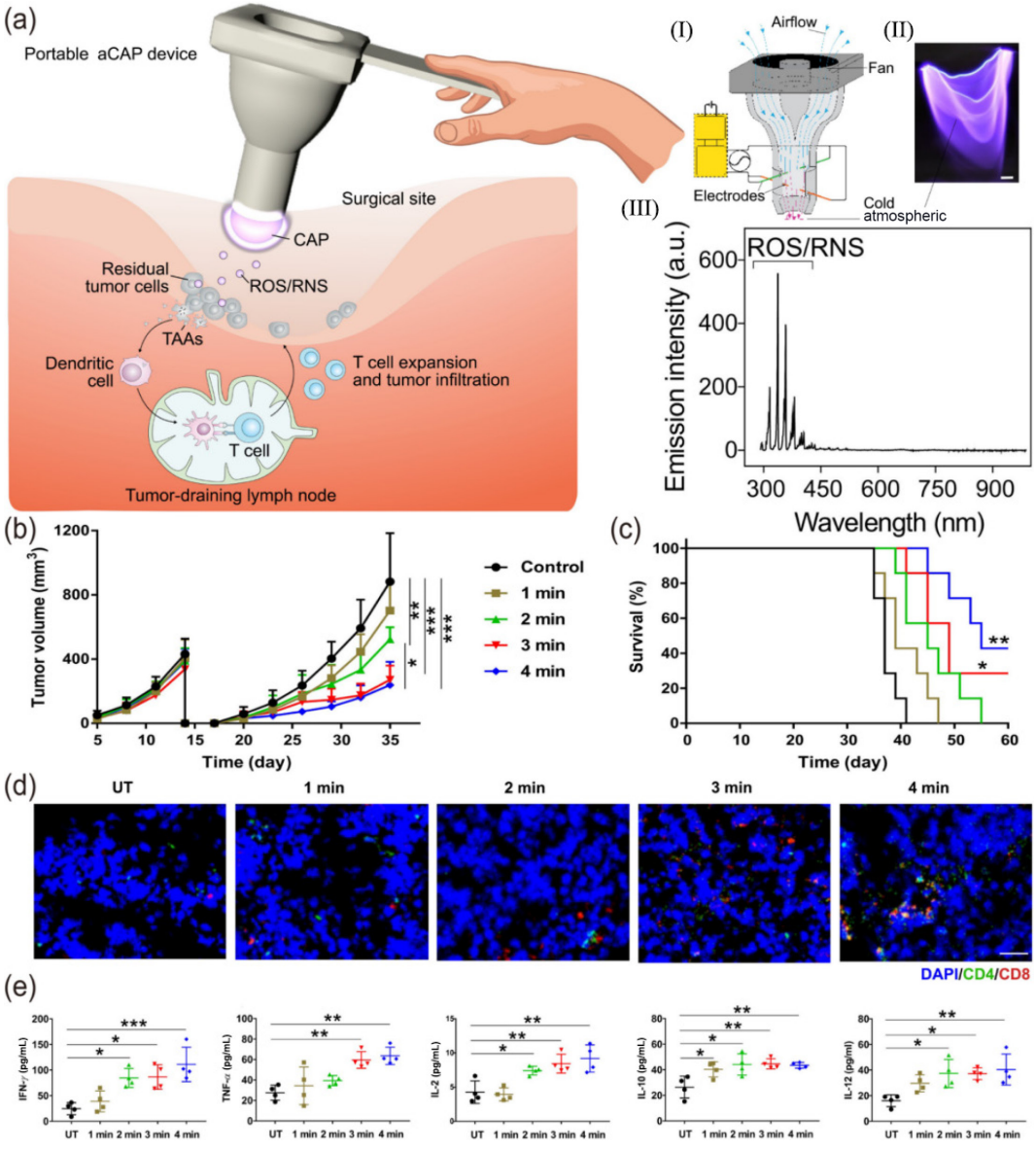

Figure 6 Utilizing a portable air-fed CAP device for postsurgical cancer treatment. (a) Illustration of portable aCAP device for postsurgical cancer treatment: (I) schematic of the working mechanism for the aCAP device; (II) an image of cold plasma discharge displaying joule-scale lightning features generated by the device, and (III) an optical emission spectrum of CAP produced by the aCAP apparatus. (b) Average tumor growth kinetics in experimental groups. (c) Kaplan-Meier survival curves for treated and control mice. (d) Immunofluorescent visualization of representative $CD4^+$ and $CD8^+$ T-cell populations within tumor tissues. (e) Cytokine levels in the serum from mice isolated 5 days after different treatments [61]. Reproduced with permission from Ref. [61], © Chen, G. et al. some rights reserved; exclusive licensee American Association for the Advancement of Science 2021.

survival pathways, including glycolysis and proliferation [140].

In the practice of combining CAP with ICB therapy, optimizing the administration route can enhance therapeutic efficacy. Chen et al. employed a hollow-structured microneedle (hMN) patch to combine CAP with ICB for percutaneous delivery, aiming to suppress the growth of both primary and distant tumors [79]. An hMN patch served as a microchannel, enabling effective transdermal delivery of CAP to interact with tumor tissues. CAP-induced cancer cell death released TAAs, which promoted the maturation of DCs in tumor-draining lymph nodes. These DCs could present major histocompatibility complex peptides to T cells, initiating a T-cell-mediated immune response. Additionally, an aPDL1, an ICB loaded within the hMN patch, was released from the hMN patch, further enhancing both local and systemic anticancer immunity. This localized therapeutic strategy may also minimize the systemic side effects associated with ICB.

The PAH is closely related to the inhibition of tumor cell growth and recurrence, as well as the regulation of the TIME by plasma. Fang et al. prepared an injectable therapeutic hydrogel, aPD-1@CAP-gel, by dissolving the anti-programmed death-1 antibody (aPD^-1), an immune checkpoint inhibitor, in a CAP-activated water and Pluronic mixture (Fig. 8(a)(I)). When administered by intratumoral injection, the ROS/RNS released by aPD-1@CAP-gel effectively induce ICD in tumors, release TAAs *in situ*, and significantly enhance the body's immune response to tumors. This includes promoting the maturation of dendritic cells, increasing the infiltration of $CD4^+$ and $CD8^+$ T cells, and polarizing macrophages from the immunosuppressive M2 phenotype to the immune-

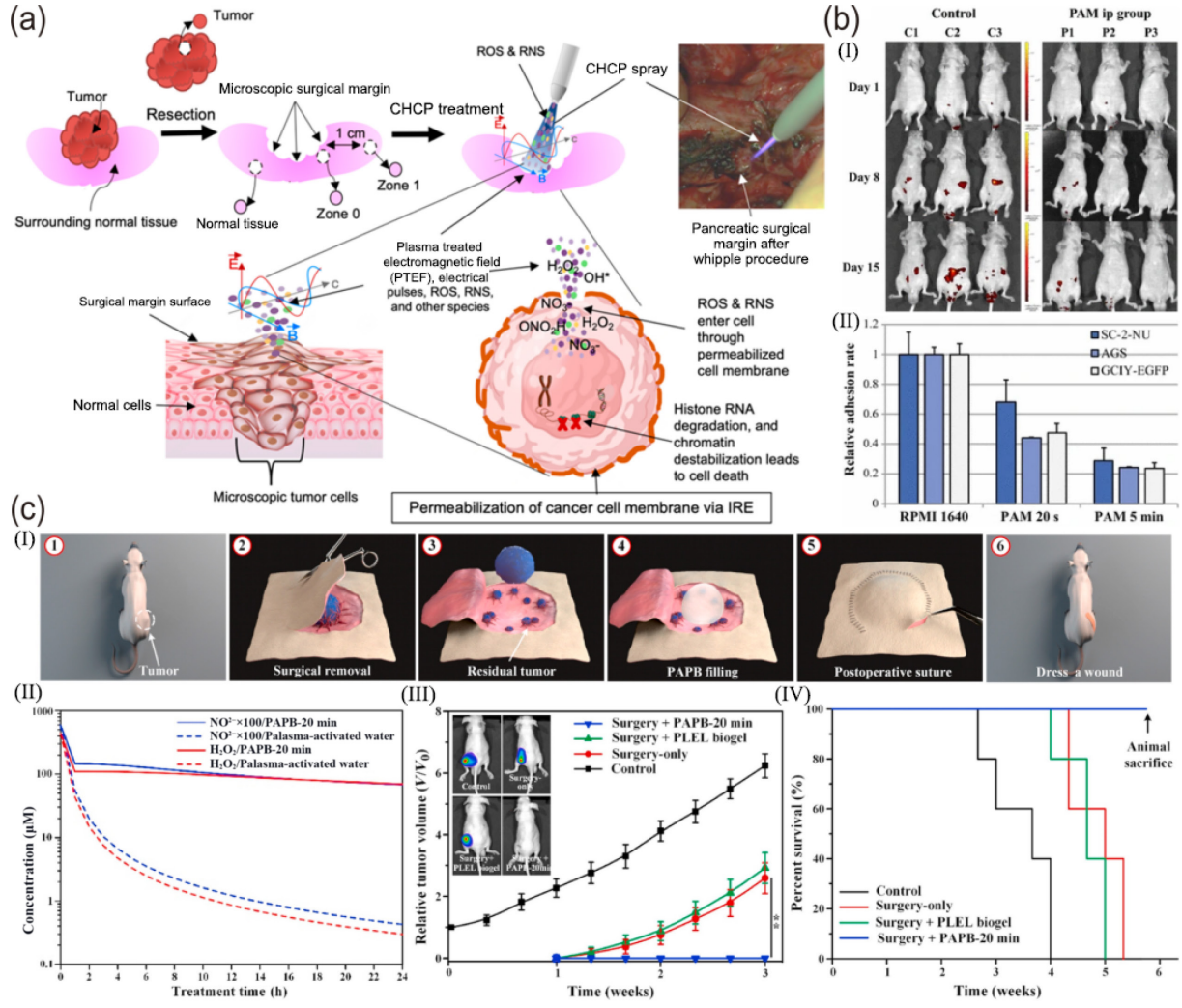

Figure 7 Postoperative plasma treatment to suppress tumor recurrence. (a) Schematic images of the surgery and CHCP treatment procedure [179]. Reproduced with permission from Ref. [179], © Canady, J. et al. Licensee MDPI, Basel, Switzerland 2023. (b) The impact of intraperitoneal injection of PAM on peritoneal metastasis of gastric cancer: (I) fluorescence images evaluating the effects of intraperitoneal injection of PAM in a mouse model of peritoneal dissemination; (II) adhesion assay of suspended cancer cells following short-term exposure to PAM [220]. Reproduced with permission from Ref. [220], © Society of Surgical Oncology 2017. (c) *In vivo* PAPB for post-operative cancer treatment: (I) schematic of surgical and post-operative PAPB treatment for the tumor; (II) simulation results of the H_2O_2 and NO_2^- concentrations on PAPB-20 min and plasma-activated water; (III) typical whole-body fluorescent images and corresponding tumor growth curves of untreated, surgery-only, surgery + PLEL, and surgery + PAPB treated tumor-bearing mice; and (IV) survival of tumor-bearing mice after different treatments [73]. Reproduced with permission from Ref. [73], © Elsevier Ltd. All rights reserved 2021.

supportive M1 phenotype, thereby enhancing antigen presentation. In addition, aPD-1@CAP-gel also increases the levels of pro-inflammatory cytokines, inhibit the secretion of anti-inflammatory cytokines, and activate T cell-mediated antitumor immunity (Fig. 8(a)(II)). The released aPD-1 further enhances T cell activity by blocking the programmed cell death protein 1 (PD-1)/programmed cell death 1 ligand 1 (PD-L1) pathway, achieving synergy between CAP and ICB. This simple local treatment strategy can successfully activate a robust innate and adaptive, local and systemic anti-tumor immune response to combat distant and metastatic diseases [82].

Because CAP irradiation can produce ROS and trigger the presentation of TAAs, plasma may be a good method to transform the cold immune environment within tumors into a "hot" immune environment. To this end, Byun et al. developed a CAP-responsive hydrogel that can store RONS and deliver nanoadjuvants to enhance tumor cell elimination and TME remodeling (Fig. 8(b)). The hydrogel consists of a hyaluronic acid-tyramine conjugate (HAT) with ROS-responsive properties, mixed with transforming growth factor-β kinase inhibitor (TRKIs)-loaded lipid nanoparticles (TLNs). CAP irradiation promotes HAT cross-linking via phenol polymerization, preserving ROS/RNS and encapsulating TLNs to form a CAP-induced hydrogel (TLN@CHG). TLN@CHG can promote the phagocytosis of tumor-specific antigens (TSA) by DCs and activate the presentation of tumor antigens by the TLN nanoadjuvants, thereby remodeling the TIME. It achieves two goals simultaneously: creating an "active" tumor environment where immune cells are in an activated state and ensuring the sustained release of TSA [84].

Interestingly, the RONS produced by plasma can also function as natural adjuvants in eliciting anticancer immunity. The Bekeschus group employed CAP technology to generate multiple RONS for oxidizing two melanoma-associated antigens, melanoma antigen recognized by T-cells (MART) and premelanosome protein

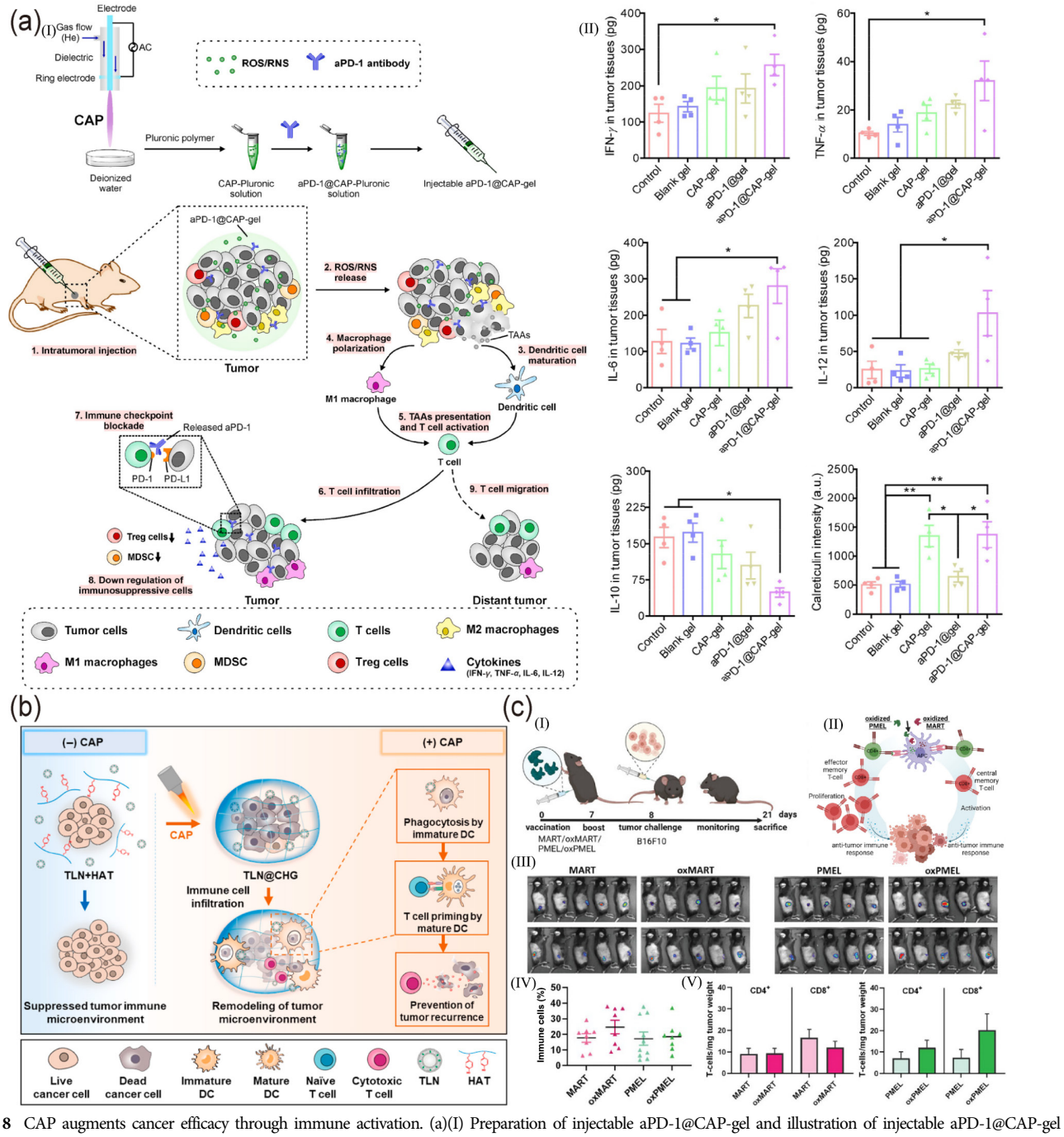

Figure 8 CAP augments cancer efficacy through immune activation. (a)(I) Preparation of injectable aPD-1@CAP-gel and illustration of injectable aPD-1@CAP-gel mediated cancer immunotherapy; (II) immunological analyses in orthotopic TNBC mice after aPD-1@CAP-gel treatment [82]. Reproduced with permission from Ref. [82], © Elsevier Ltd. All rights reserved 2023. (b) CAP treatment can achieve *in vivo* gelation of TLN@CHG, which acts as an antigen reservoir to reshape the TIME, promoting the recruitment, maturation, and activation of DC, and ultimately preventing tumor recurrence through a systemic immune response [84]. Reproduced with permission from Ref. [84], © Elsevier Ltd. All rights reserved 2023. (c) Immunization of murine models with CAP-oxidized TAAs elicited a tumor-suppressive immune response: (I) the impact of oxidation on the immunogenicity of MART and PMEL was assessed via an *in vivo* rechallenging assay; (II) hypothesis of two modes of action after vaccination with oxMART and oxPMEL; (III) representative *in vivo* luminescent imaging of anesthetized mice was conducted to monitor tumor growth dynamics post-immunization with MART/oxMART or vaccination with PMEL/oxPMEL; (IV) a percentage of tumor-infiltrated $CD45^+CD3^+$ and $CD45^+IAIE^+$ immune cells; (V) $CD4^+$ helper cells and cytotoxic $CD8^+$ in tumors after MART, oxMART vaccination or PMEL, and oxPMEL vaccination [221]. Reproduced with permission from Ref. [221], © Clemen, R. et al. Advanced Science published by Wiley-VCH GmbH 2024.

(PMEL). This process induced oxidative post-translational modifications (oxPTMs) on the proteins, enhancing immune cell-mediated tumoricidal activity (Figs. 8(c)(I)–8(c)(III)). The results demonstrated that vaccinating mice with oxidized MART or PMEL (oxMART, oxPMEL) before live melanoma cell challenge significantly altered cytokine secretion profiles and promoted T cell differentiation among tumor-infiltrating leukocytes (Figs. 8(c)(IV) and 8(c)(V)). Specifically, oxMART stimulated the activation of cytotoxic central memory T cells, while oxPMEL induced the proliferation of cytotoxic effector T cells. These findings suggest that

CAP-mediated oxidative modification of melanoma-associated antigens is crucial for eliciting anti-cancer immunity [221].

In a nutshell, CAP is widely recognized as a genuine inducer of ICD. Following ROS-induced cell demise, it triggers the expression and release of damage-associated molecular patterns (DAMPs), which attract antigen-presenting cells to the tumor site. This facilitates efficient antigen processing and presentation by antigen-presenting cells, thereby sensitizing cancer cells to multiple therapeutic modalities. Consequently, localized tumor oxidation via medical plasma technology serves as a therapeutic sensitizer in oncology, enhancing tumor responsiveness to diverse treatment strategies [23].

4.1.8 Synergistic cancer therapy: Combining CAP with other drugs

CAP can be used in combination with targeted antitumor drugs as well. The combination of CAP and cetuximab can suppress the invasion and migration of cetuximab-resistant oral squamous cancer cells by inhibiting the NF-κB pathway [222]. This approach may offer a promising strategy to overcome resistance to anti-epidermal growth factor receptor (anti-EGFR) therapies. CAP exhibits synergistic effects with various cancer treatment modalities and is not restricted to use in combination with a single therapy. This broad potential for synergy is likely based on multiple mechanisms, including the elevation of free radical levels, induction of DNA damage or genomic instability in cancer cells, and alteration of key signaling factor expression through epigenetic DNA modifications, rendering tumor cells more susceptible to the effects of other drugs [180].

Additionally, combining CAP with nanomedicine can achieve enhanced therapeutic outcomes. For instance, CAP-based treatment in conjunction with iron oxide magnetic nanoparticles induced apoptosis in A549 lung cancer cells by modulating epithelial-mesenchymal transition (EMT) and inhibiting EGFR-PI3K/AKT and EGFR-RAS/ERK signaling pathways, thereby more effectively suppressing tumor growth [87]. Zhang et al. developed a novel dual-modal cancer therapy targeting A549 by integrating CAP with a new paclitaxel-based nanomedicine. *In vitro* studies revealed that the synergistic effect between the novel paclitaxel nanomedicine and CAP promoted intracellular ROS generation, leading to significant apoptosis of A549 cells. These findings indicate that this combinatorial approach exerts a more pronounced inhibitory effect on A549 cell proliferation [88].

In summary, CAP can be used alone or in combination with other therapies. It effectively clears cancer cells, reduces tumor drug resistance, and enhances antitumor immune responses through a variety of different mechanisms, making it a promising adjuvant therapy for cancer. These mechanisms include the induction of immunogenic cell death in cancer, the introduction of oxPTMs to proteins and peptides, epigenetic modification of aberrant gene expression, and the enhancement of the anti-tumor functions of immune cells [56]. Moreover, by modulating cellular redox homeostasis, CAP can serve as a potential sensitizer of the TME, an inducer of ICD, and a killer of cancer stem cells (CSC) [223].

4.2 CAP in autoimmune disorders

Autoimmune diseases are a heterogeneous group of chronic diseases; though considered relatively rare, they can significantly incur mortality and morbidity [224, 225]. The overall prevalence of autoimmune diseases is approximately 3%–5% [226]. Currently, the management of autoimmune diseases predominantly relies on pharmacotherapy, encompassing systemic immunosuppression, nonsteroidal anti-inflammatory drugs, and glucocorticoid therapy. While these treatments can alleviate symptoms, they often result in suboptimal therapeutic responses among many patients [227, 228].

Furthermore, immunomodulatory drugs, due to their broad and nonspecific effects, can readily trigger adverse events such as infections and malignancies, often accompanied by long-term adverse reactions that may lead to disability, reduced lifespan, and complications [229]. Autoimmune diseases are typically linked to immune hyperactivity, as antibodies produced by the immune response attack self-tissues rather than combating infections. This process results in inflammation, and thus, autoimmunity is often marked by an inflammatory environment [9]. Given CAP's redox-regulating attributes, its potential for treating autoimmune and inflammation-mediated diseases is suggested, which may broaden its clinical utility [230].

4.2.1 CAP therapy for rheumatoid arthritis: Mechanisms and therapeutic potential

Rheumatoid arthritis (RA), a chronic autoimmune disorder, involves synovial inflammation, cartilage and bone destruction, and systemic inflammation, resulting in physical disabilities and joint deformities [231, 232]. This disease is associated with extensive clinical sequelae, including comorbidities that predominantly impact the skeletal system, vasculature, cognition, and metabolic function. As the most common inflammatory arthritis, RA is currently incurable and is considered a lifelong condition [233]. Hypoxia is implicated in RA pathogenesis, as synovial hypoxia induces inflammatory cells to trigger invasive mechanisms, accelerating the proliferation and migration of rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS). Activated RA-FLS cells are central to the pathogenesis of synovial proliferation in RA, exhibiting pathological characteristics similar to cancer cells. Furthermore, given the tumorigenic properties of the synovium, therapeutic approaches for tumor diseases may hold promise for RA treatment [234]. Therefore, utilizing CAP to selectively target RA-FLS with increased mitotic activity represents a promising therapeutic strategy. Ding et al. demonstrated that 120 s of CAP treatment significantly reduced RA-FLS cell viability, elevated intracellular ROS concentrations, and markedly decreased the Bcl-2/Bax protein ratio associated with apoptosis. These results provide a theoretical basis for CAP-targeted therapy in RA [235]. Subsequently, Faramarzi et al. discovered that CAP reduced cell viability by generating ROS, increasing the percentage of apoptotic RA-FLS. Additionally, CAP significantly diminished the production of inflammatory cytokines, such as NF-κB and interleukin 6 (IL-6), and destructive factors like receptor activator of nuclear factor kappa-B ligand and matrix metalloproteinase-3. This suggests that CAP can decelerate the progression of RA by mitigating the tumorigenic characteristics and inflammatory responses of RA-FLS [236].

CAP therapy for RA has demonstrated significant efficacy both at the cellular level and in animal model experiments, where notable therapeutic effects were observed. Ding et al. conducted both *in vivo* and *in vitro* studies, demonstrating that appropriate doses of CAP significantly mitigated symptoms such as synovial proliferation, inflammatory infiltration, and angiogenesis in RA. Ding et al. integrated *in vitro* experiments and *in vivo* arthritic animal models to show that optimized CAP dosing alleviated RA-related pathologies, including synovial hyperplasia, inflammatory

cell infiltration, and neovascularization. This therapeutic effect of CAP stems from its capacity to reverse RA pathology by regulating tissue stress responses and boosting endogenous antioxidants. Specifically, CAP triggers apoptosis in RA-FLS by activating the mitochondrial apoptotic pathway, including increasing intracellular ROS levels, disrupting the Bcl-2/Bax expression ratio, damaging mitochondrial membrane potential, and activating downstream caspase-3 (Fig. 9(a)) [57]. Ding et al. further confirmed the therapeutic potential of direct CAP application on synovial tissue in rats with adjuvant-induced arthritis (AIA). They surgically exposed the knee joint synovium and treated the hyperplastic synovial tissue using a DBD plasma device (Fig. 9(b)). The results showed that CAP triggered apoptosis in RA-FLS cells while simultaneously activating the antioxidant capacity of surrounding tissues. After CAP treatment, the absence of synovial hyperplasia, angiogenesis, and inflammatory infiltration significantly ameliorated RA symptoms [58]. These findings suggest a potential minimally invasive and targeted therapeutic approach for RA.

4.2.2 CAP therapy for psoriasis: Mechanisms and therapeutic potential

Psoriasis is a chronic inflammatory skin disease linked to conditions like depression, psoriatic arthritis, and cardiometabolic syndrome [237]. This global autoimmune disorder can occur at any age, imposing a significant burden on patients and society [238]. The WHO defines it as a "chronic, non-communicable, painful, disfiguring, and disabling disease with no cure" [239]. The cutaneous manifestations of psoriasis are characterized by marked hyperproliferation of keratinocytes and differentiation alterations associated with leukocyte infiltration in both the dermis and epidermis, which are primarily triggered and maintained by T lymphocytes [240]. As an emerging therapeutic approach, CAP treatment addresses psoriasis through immunomodulation, inflammation suppression, and promotion of keratinocyte apoptosis, while also reducing the use of traditional therapeutic drugs and mitigating the adverse reactions and toxic side effects that may arise from long-term medication. Zhong et al. found that appropriately dosed DBD surface air plasma could induce apoptosis in keratinocytes and modulate the release of psoriasis-related cytokines in a dose-dependent manner. It also effectively inhibited the hyperproliferation of keratinocytes and amplification of inflammation by inactivating IL-12 and activating T cells [241]. Notably, Klebes et al. assessed the therapeutic efficacy of tissue-tolerant plasma (TTP) in a cohort of six psoriasis patients. The research indicated that while TTP demonstrated some therapeutic benefits for psoriasis and a temporary reduction in bacterial colonization on skin lesions, its efficacy in managing psoriatic plaques did not surpass that of conventional treatments [242]. This underscores the need for researchers to develop more effective CAP therapies and delivery methods. Kim et al. developed a CAP patch using flexible polymer films through surface dielectric barrier discharge (SDBD) technology (Fig. 10(a)) [60]. This CAP patch can uniformly deliver ROS/RNS from plasma to the skin, opening calcium channels and inducing calcium influx in keratinocytes. By doing so, it inhibits abnormal differentiation, reduces inflammation-related cytokines, and suppresses the disruption of tight junctions, thereby significantly alleviating psoriasis symptoms. The therapeutic effect is primarily due to the generation of ROS/RNS by the CAP patch, rather than the electric field, and it exhibits no specific toxicity. Additionally, the CAP patch is portable, safe, and convenient. Owing to the distinct characteristics between psoriatic and non-psoriatic skin areas, the CAP patch can be specifically applied to the lesion areas, making it easy to combine with existing medications. This not only potentially reduces drug side effects but also promises to decrease the drug dosage.

To fully realize the clinical potential of CAP for psoriasis treatment, it is essential to develop an effective transdermal delivery method to augment its efficacy in inducing oxidative stress and apoptosis in keratinocytes. Wu et al. designed a novel Plasma-Activated Ice Microneedle (PA-IMN) patch that can contain and enable localized transdermal delivery of RONS, offering an alternative to direct CAP irradiation therapy [72]. This patch, optimized through freezing treatment for enhanced mechanical properties, can effectively penetrate the thickened stratum corneum

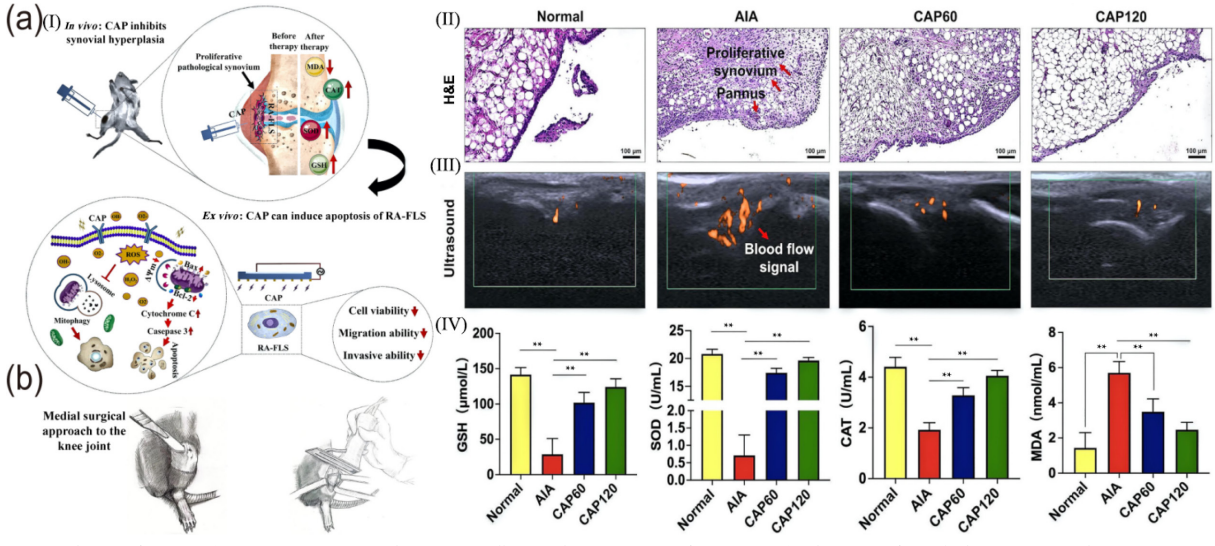

Figure 9 CAP therapy for RA. (a) *In vivo* treatment with CAP can alleviate the symptoms of AIA rats: (I) schematic of CAP's therapeutic mechanism on AIA rat joint synovium *in vivo* and *ex vivo*; (II) changes in H&E staining of the synovial tissue of the knee joint in each group of rats; (III) ultrasound blood flow changes in the knee joint synovial membrane of rats; (IV) oxidative stress indices before and after treatment [57]. Reproduced with permission from Ref. [57], © Ding, C. et al. Bioengineering & Translational Medicine published by Wiley Periodicals LLC on behalf of American Institute of Chemical Engineers 2022. (b) CAP-DBD-Joint *in vivo* experimental effect diagram [58]. Reproduced with permission from Ref. [58], © Published by Elsevier B.V. 2023.

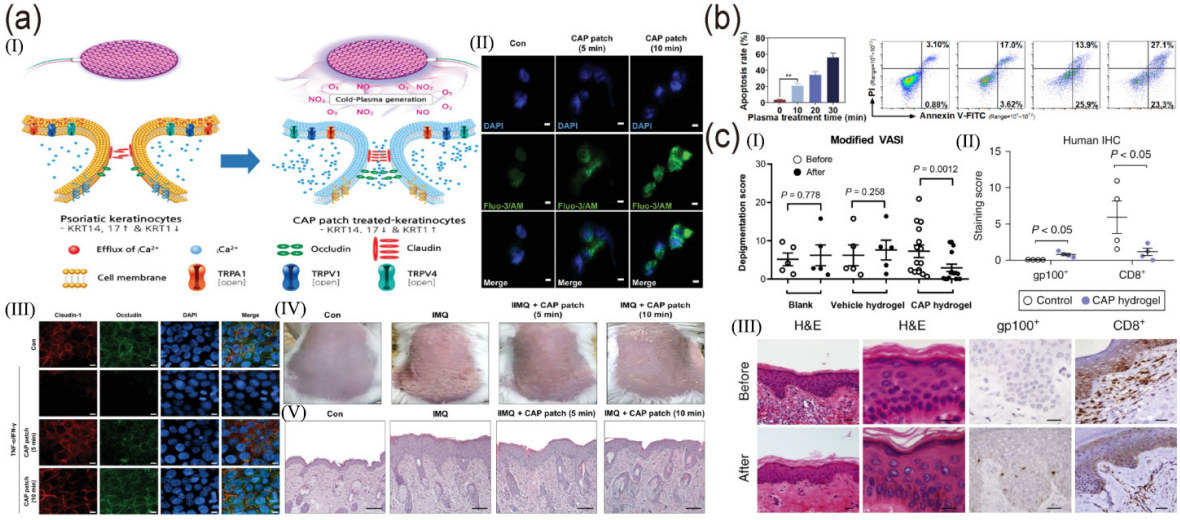

Figure 10 CAP therapy for psoriasis and vitiligo. (a) Portable CAP patch-mediated psoriasis therapy: (I) schematic of CAP patch's therapeutic mechanism in psoriasis. CAP patch induced the influx of Ca^{2+} via calcium channels decreased psoriasis-associated gene expression and protein levels, induced normal keratinocyte differentiation, and inhibited the disruption of tight junctions in keratinocytes; (II) calcium image of intracellular calcium influx of keratinocytes by CAP patch; (III) fluorescence microscopic images of immunostained tight junctions in HaCaT cells treated with TNF-α/IFN-γ or CAP patch; (IV) phenotypic observations of dorsal skin; (V) mouse skin tissues stained with H&E to perform histological observation [60]. Reproduced with permission from Ref. [60], © Kim, N. et al. Advanced Science published by Wiley-VCH GmbH 2022. (b) HaCaT apoptosis rates at 24 h post-treatment with distinct PA-IMN patches [72]. Reproduced with permission from Ref. [72], © American Chemical Society 2024. (c) Histologic changes after CAP-activated hydrogel treatment: (I) modified VASI (vitiligo area scoring index) scores in different groups are shown; (II) the scores of $gp100^+$ and $CD8^+$ cells were measured in the blank control and CAP hydrogel groups; (III) melanocytes and melanin particles were observed in H&E-stained sections. Using immunohistochemistry, $gp100^+$ and $CD8^+$ T cells were observed in the epidermis and underneath the epidermis, respectively. Reproduced with permission from Ref. [74], © Zhai, S. et al. Published by Elsevier, Inc. on behalf of the Society for Investigative Dermatology 2021.

and deliver active substances to deep tissues. The RONS released by PA-IMN cause mitochondrial dysfunction and apoptosis in keratinocytes by elevating ROS levels, effectively inhibiting the hyperproliferation of keratinocytes (Fig. 10(b)). Additionally, PA-IMN effectively suppress inflammatory cytokine release, mitigate psoriasiform inflammation, and demonstrate therapeutic efficacy in imiquimod-induced murine dermatitis, without obvious systemic toxicity observed. As an efficient transdermal delivery platform, PA-IMN amplify oxidative stress-driven apoptosis in keratinocytes, consequently suppressing epidermal hyperplasia and downstream inflammatory responses. They show great clinical potential in treating psoriasis while maintaining high biosafety without damaging major organs.

4.2.3 CAP therapy for vitiligo: mechanisms and therapeutic potential

CAP therapy has been experimentally validated to attenuate the cutaneous expression of lymphocyte function-associated antigen 1 (LFA-1) and intercellular adhesion molecule 1 (ICAM-1) in psoriasis mouse models, adhesion molecules that are pathologically upregulated in vitiligo lesions [243]. Inspired by this, Zhai et al. exploratively employed PAH to treat vitiligo-like mouse models and skin lesions in vitiligo patients, achieving significant clinical results. The underlying therapeutic mechanisms may involve reducing the infiltration of immune cells, particularly the number of $CD11c^+$ dendritic cells, $CD3^+$ T cells, and $CD8^+$ T cells; inhibiting the release of inflammation-related transcription factors and chemokines, such as C-X-C motif chemokine ligand 10 (CXCL10), interferon-γ (IFN-γ), and hypoxia-inducible factor-1α (HIF-1α). Additionally, PAH treatment exerts therapeutic effects by modulating the oxidative stress response, specifically by downregulating iNOS expression and upregulating the transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) expression. In clinical trials involving patients with active focal vitiligo, PAH therapy demonstrated significant efficacy, and no adverse effects were observed during the entire trial period or subsequent follow-up. In a clinical trial targeting active focal vitiligo, PAH therapy demonstrated significant efficacy. VASI scores revealed substantial improvement in lesions treated with CAP hydrogel compared with baseline (Fig. 10(c)(I)). Histologically, hematoxylin and eosin (H&E) staining of untreated lesions showed complete absence of melanocytes and melanin granules, whereas CAP hydrogel treatment partially restored both (Fig. 10(c)(III)). Immunohistochemistry further confirmed a marked increase in cells expressing the melanocyte differentiation antigen glycoprotein 100, and concurrently, a pronounced reduction in the previously dense $CD8^+$ T-cell infiltrate beneath the lesional epidermis (Fig. 10(c)(II)), an outcome highly favorable for vitiligo management. Therefore, CAP therapy is established as an effective and safe new method for treating vitiligo [74].

4.3 CAP for diabetes and wound healing

Diabetes affects 463 million people globally. Among its complications, diabetic cutaneous ulcers (DCUs) occur in approximately 25% of patients and lack effective treatments [244]. DCUs carry high disability risk, with about 20% of patients facing lower-limb amputation, and improper care contributes to 13% mortality within one year of diagnosis. Impaired wound healing in diabetes is driven by multiple interrelated factors, primarily including defective angiogenesis, bacterial infection, uncontrolled inflammation, oxidative stress, and neuropathy, which mutually reinforce one another to form a vicious cycle [245]. Inadequate vascularization reduces oxygen and nutrient supply, perpetuating chronic inflammation and suppressing collagen deposition, whereas infection acidifies the microenvironment, impairs endothelial cell proliferation, and recruits inflammatory cells, thereby exacerbating

tissue damage. Additionally, hyperglycemia promotes biofilm formation, which restricts antibiotic penetration, fosters drug resistance, and ultimately culminates in healing failure [246].

Current management of DCU faces significant limitations, compounded by rising bacterial resistance, which renders monotherapy increasingly insufficient [247, 248]. Given the persistent healing difficulties and high recurrence rates, integrated strategies combining anti-infection, anti-inflammatory, and pro-angiogenic approaches are urgently needed to minimize drug toxicity and resistance risks. CAP has emerged as a promising therapy, exhibiting potent anti-infective, anti-inflammatory, and angiogenic properties that promote rapid DCU healing without inducing bacterial resistance, and demonstrates no long-term adverse effects, making it well-suited for chronic wound management [249].

4.3.1 CAP therapy for diabetic wound

In a clinical trial, low-temperature atmospheric pressure argon plasma technology exhibited the capability to significantly reduce bacterial loads in various chronic wounds. The treatment process was safe, painless, and devoid of side effects or complications, demonstrating the potential to promote wound healing. This result aligns with prior *in vitro* wound model findings using human keratinocytes (HaCaT), which demonstrated that under bacterial contamination, the mechanically induced gap closure rate significantly increased after 40 s of plasma treatment [63, 250]. Mirpour et al. performed a randomized clinical trial demonstrating that CAP therapy significantly expedites diabetic foot ulcers (DFU) healing, with an immediate yet transient antiseptic effect observed (Fig. 11(a)) [251]. Similar therapeutic efficacy was also validated through a clinical trial focused on evaluating the effectiveness of plasma therapy for chronic venous ulcers, reinforcing the consistency of these findings across distinct wound types (Fig. 11(b)) [252]. Another clinical trial, which was placebo-controlled, blinded, and prospective, applied CAP to chronic wounds related to DFU and found that it significantly accelerated wound healing and reduced wound size (Fig. 11(c)). Simultaneously, a hypothesis was proposed that the therapeutic effect of CAP does not primarily depend on its antibacterial action but rather directly activates dormant chronic wounds, suggesting that the positive impacts of CAP treatment on wound healing are multi-faceted [64].

Cheng et al. applied an argon-based atmospheric pressure plasma jet to treat chronic wounds in type 1 and type 2 diabetic rats (Fig. 11(d)). Histological examination revealed that, relative to the untreated control group, the plasma-treated group exhibited accelerated re-epithelialization, increased collagen production, reduced inflammation, and a more preserved skin architecture. Additionally, during the mid-to-late phases of the wound-healing process, a decrease in neovascularization is observed in tissues that have undergone plasma treatment. This reduction is a consequence of the fact that plasma-treated wounds display heightened TGF-β expression during the initial stages, which subsequently wanes in the mid-to-late stages. Furthermore, in the plasma-treated tissues, due to exposure to excessive ROS, the levels of antioxidants (including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT)) increased, which aided in inflammation suppression and wound healing [65]. Mirpour et al. demonstrated in clinical trials that CAP is an effective treatment for wounds in type 2 diabetics, accelerating DFU wound healing and exerting immediate antiseptic influence; however, these effects didn't seem to have a long-lasting impact [251]. He et al. discovered that 3 min of daily CAP treatment enhances wound healing in diabetic mice by reducing inflammation and oxidative stress, promoting angiogenesis, and ensuring liver and kidney safety. Both the low-dose and high-dose groups exhibited significantly higher wound healing rates compared to the control group. Additionally, compared to the control group, the protein expressions of IL-6, TNF-α, iNOS, and SOD were significantly reduced, while the protein expressions of VEGF and TGF-β were significantly increased in both treatment groups [66]. Intriguingly, Rezaeinezhad et al. demonstrated that CAP not only effectively modifies glycated GPx, enhancing its enzymatic activity, but also markedly reduces oxidative stress levels, concentrations of advanced glycation end-products (AGEs), and inflammatory cytokine levels in diabetic mice. Based on these findings, they put forward the idea that CAP therapy can act as a new adjuvant option. It should be combined with metformin for the treatment of diabetes, aiming to lower the metformin dose and reduce its potential negative impacts [39].

4.3.2 CAP therapy for other chronic wounds

The global aging population has led to an increase in the number of patients with chronic wounds other than diabetic wounds. This growth has imposed a significant burden on the healthcare system [254]. Fortunately, plasma is not only applied in the treatment of diabetic wounds but also plays a crucial role in the care of other types of chronic wounds, such as pressure ulcers and venous leg ulcers. Chatraie has utilized CAP for treating pressure ulcers. The study findings reveal a multi-faceted enhancement in wound healing outcomes with plasma treatment compared to the control group. Firstly, regarding mechanical properties, the wounds in the plasma-treated group demonstrated a significantly higher mechanical strength during the healing process than those in the control group. Secondly, from a histological perspective, re-epithelialization, a crucial step in wound healing, occurred at a markedly accelerated rate in the plasma-treated group when compared to the control group. Moreover, plasma treatment exerted a profound impact on the physiological processes of wound healing. It substantially promoted angiogenesis (the formation of new blood vessels) and fibrosis (the deposition of connective tissue), which are vital for tissue repair [67]. Moreover, a clinical trial utilizing a handheld DBD plasma generator for treating chronic lower limb venous ulcers indicated that CAP effectively suppressed bacterial load at the wound site, reduced pain, and promoted wound healing [204]. Further investigations have revealed that plasma therapy for venous ulcers can diminish exudate and bacterial load, foster tissue regeneration, accelerate wound healing, and leave no signs of infection [252]. In addition, CAP therapy typically being non-invasive and non-contact necessitates no specific anesthesia. Conversely, this treatment may alleviate pain caused by ulcers or traditional wound dressings, and plasma therapy can further reduce pain, itchiness, and odor associated with skin ulcers [255]. Therefore, it is evident that CAP can be an effective treatment for chronic wounds.

4.3.3 Mechanisms and therapeutic potential of CAP in chronic wound healing

The mechanisms underlying the above-described therapeutic effects of CAP in chronic wound healing can be summarized as follows. A key advantage of CAP over traditional drug treatments for chronic wounds is its broad-spectrum antibacterial effects [100]. During direct CAP treatment, ·OH and $^1O_2$ play pivotal roles in inactivating

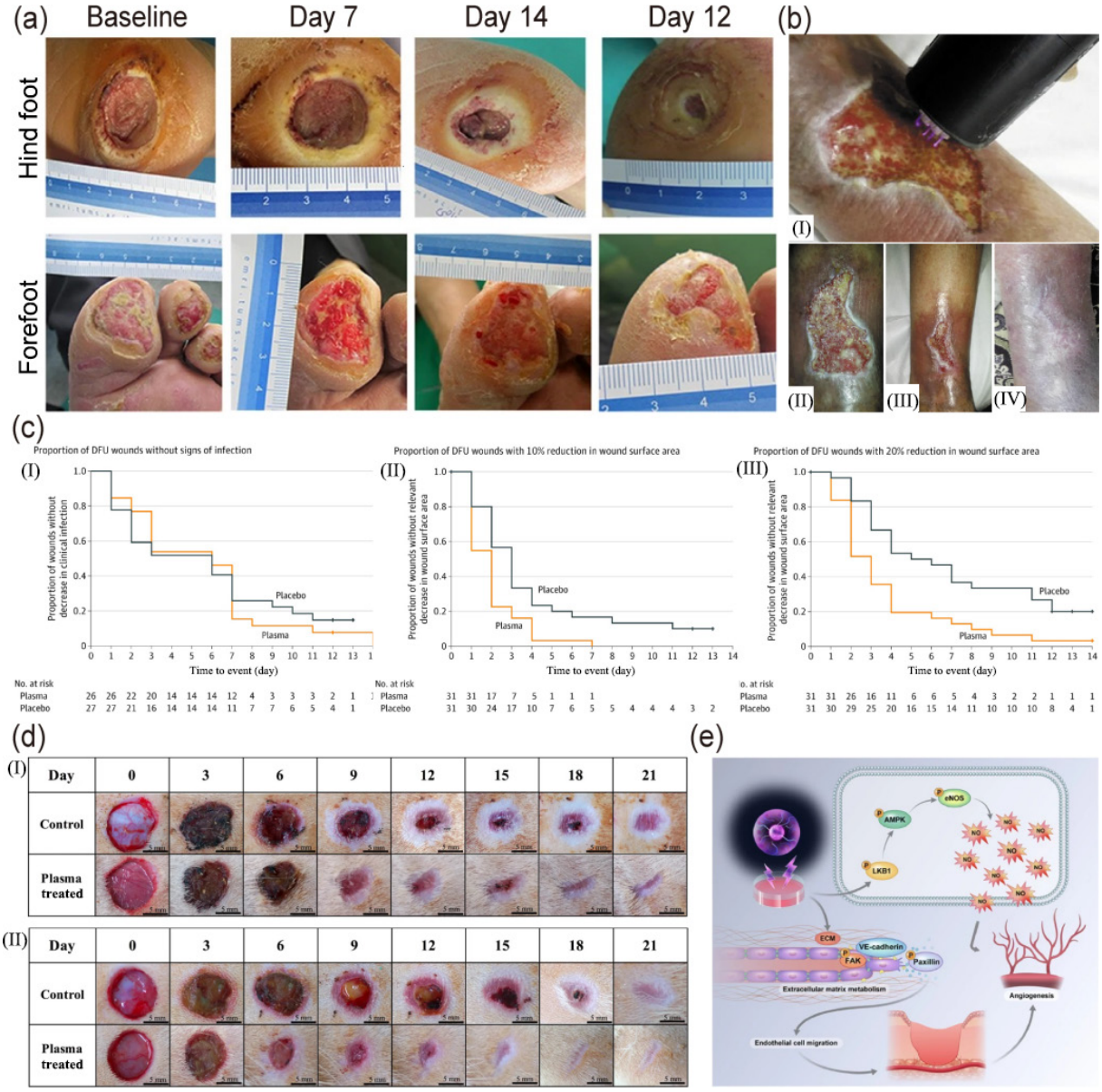

Figure 11 Plasma treatment of chronic wounds. (a) Wound contraction in two plasma-treated cases after 3 weeks [251]. Reproduced with permission from Ref. [251], © Mirpour, S. et al. 2020. (b) CAP Jet treating human chronic venous ulcer: (I) moderate exudate, and sloughy tissue at the baseline treated with CAP-jet; (II) wound 2 weeks after the beginning no exudate, and granulation tissue; (III) the wound 4 weeks after the start is entirely healed without any signs of infection [252]. Reproduced with permission from Ref. [252], © Samsavar, S. et al. Published by S. Karger AG, Basel 2022. (c) (I) The proportion of wounds without signs of clinical infection during treatment; (II) the proportion of wounds achieving a 10% reduction in wound surface area during treatment; (III) the proportion of wounds attaining a 20% reduction in wound surface area during treatment [64]. Reproduced with permission from Ref. [64], © Stratmann, B. et al. (d) The temporal variations in wound dynamics of small wounds at distinct time intervals during the healing process in diabetic groups undergoing plasma jet treatment: (I) type 1 diabetic wound and (II) type 2 diabetic wound [65]. Reproduced with permission from Ref. [65], © Cheng, K.-Y. et al. 2018. (e) Liquid plasma promotes angiogenesis through upregulation of endothelial nitric oxide synthase-induced extracellular matrix metabolism [253]. Reproduced with permission from Ref. [253], © Kang, S. U. 2024.

fungi by disrupting the integrity of cellular walls and membranes. In contrast, PAW inhibits fungal energy metabolism through NO, causing more fungi to enter a viable but non-culturable state. The synergistic effect of continuous CAP and PAW application is due to elevated intracellular concentrations of $^1O_2$ and $ONOO^-$. These RONS, in conjunction with membrane damage, cause irreparable oxidative damage to mitochondria and the energy metabolism system, thereby further promoting wound healing [256].

Beyond direct antimicrobial effects, CAP and PAW also promote wound healing through modulation of endothelial function, cell proliferation, and inflammatory responses. Kang et al.'s research shows that PAW treatment significantly enhances the level of endothelial nitric oxide synthase-adenosine monophosphate-activated protein kinase (eNOS-AMPK) and intracellular NO levels (Fig. 11(e)). Since AMPK plays a crucial role in regulating endothelial function and metabolism and is known to stimulate endogenous NO production to further increase blood flow, PAW treatment promotes the migration of human umbilical vein endothelial cells (HUVECs) and the activation of extracellular matrix (ECM) metabolism by activating AMPK and inducing NO production. This, in turn, enhances microcirculation, including the transport of nutrients, leukocyte migration, and oxygen delivery, to

counteract hypoxia and promote wound healing [253]. Similarly, Barton et al. found that CAP treatment of HaCaT cells upregulates wound healing-related factors, including VEGF, FGF, HBEGF, and IL-6, which play key roles in angiogenesis and proliferation, indicating that CAP facilitates healing by augmenting ROS/RNS levels [257]. Schmidt et al. further demonstrate that CAP treatment for wounds correlates with the activation of multiple physiological processes. These include enhanced infiltration of macrophages and neutrophils, upregulation of transcription of both pro-inflammatory and anti-inflammatory mediators, increased proliferation and decreased apoptosis, stimulation of Nrf2-driven antioxidant defense responses, and enhanced angiogenesis. Through experiments, they have proved that gas plasma treatment can also alter the phosphorylation of signaling molecules in adhesion-related complexes and affect the transcription of cell surface adhesion receptors, structural proteins, and marker genes related to cell differentiation. Additionally, Schmidt et al. have proven that gas plasma can increase tissue oxygenation and skin perfusion to achieve ROS-driven wound healing, fully confirming the synergistic effect of CAP in stimulating healing rather than being dominated by a single effect [258, 259].

CAP also can regulate wound inflammatory responses. Specifically, PAW can promote a balanced release of pro-inflammatory and anti-inflammatory factors within mouse wounds, thereby accelerating the resolution of inflammation in the treated group and promoting their early entry into subsequent stages in wound-repair progression. In comparison with the control group, the PAW-treated group demonstrated a marked upregulation in the expression levels of VEGF within the wound sites, indicating that microvascular proliferation around the wounds in this group was faster, thereby expediting the progression of wound repair [260]. Furthermore, chronic wounds often accompany repeated micro-injuries and bleeding, especially during the process of debridement to remove necrotic tissue and excess material, which can easily trigger bleeding. CAP's ability to promote coagulation exerts a crucial influence in this context [261]. Bekeschus believes that CAP-induced platelet activation is primarily a secondary reaction, and hemolysis is a necessary and sufficient condition for CAP-induced platelet activation. Following hemolysis, erythrocytes release several DAMPs, such as hemoglobin, heme, and adenosine diphosphate. These DAMPs, together with ROS generated by CAP, mediate platelet activation, ultimately leading to hemostasis [262].

In summary, the etiology of chronic wounds is complex, but most ulcers are caused by ischemia, diabetes-related complications, venous insufficiency, or pressure [40]. In the clinic, methods for managing and treating chronic wounds are limited, often addressing only a single underlying factor. Common approaches include manual debridement, the use of anti-inflammatory drugs, or pharmaceutical dressings to prevent wound infection and promote healing. However, almost all chronic wounds are colonized by biofilms, and the types of bacteria can change due to individual patient differences and disease progression, increasing the risk of bacterial resistance. When the growth of one or more bacteria exceeds a critical threshold and causes pathological damage, it transitions to an infected state [246]. For infected cases, systemic antibiotic therapy is frequently employed to control the infection and aid wound healing [263]. However, if the infection cannot be effectively contained and other treatments fail, amputation may be required, severely impacting the patient's quality of life and life expectancy. In this context, CAP serves as a novel therapeutic approach and has demonstrated significant efficacy in addressing the pathological aspects of chronic wounds, such as bacterial infections, excessive oxidative stress, macrophage dysfunction, and inflammation exacerbation, exhibiting great therapeutic potential [40].

4.4 CAP for potential treating neurological disorders

Treating traumatic central nervous system injuries and neurodegenerative diseases remains a major medical challenge, while the application of neural stem cells (NSCs) offers novel therapeutic strategies for these conditions [44]. Given that modulating intracellular redox status is essential for determining cell fate, ROS participate in regulating transcriptional events leading to proliferation and differentiation in both mammalian and plant cells. Thus, CAP can be used to enhance NSC growth and development, which is highly significant for managing neurodegenerative diseases such as Alzheimer's disease and traumatic central nervous system injuries (Fig. 12(a)) [264]. The therapeutic principle of CAP involves complex physicochemical and biological interactions, wherein NO serves as an upstream extracellular messenger, synergizing with mitochondrial superoxide radicals ($O_2^{\cdot-}$) and cytosolic H_2O_2 as reactive oxygen messengers, playing a crucial role in CAP-induced neural differentiation. Specifically, under the effect of CAP, excited atomic oxygen in the plasma state leads to the formation of RONS within the physicochemical and biological (PCB) network.

These RONS react with active atoms in the extracellular fluid to produce NO. NO further induces reversible inhibition of mitochondrial complex IV, generating a large number of $O_2^{\cdot-}$, which then mutate into cytosolic hydrogen peroxide, serving as an intracellular messenger to specifically activate the Trk/Ras/ERK signaling pathway, thereby inducing neural differentiation (Fig. 12(b)(I)) [62]. The therapeutic principles of plasma treatment for neurological disorders have undergone preliminary validation in live zebrafish experiments (Figs. 12(b)(II))–12(b)(IV)). Compared with traditional treatment methods (such as using retinoic acid, resveratrol, or serum starvation), CAP-enhanced NSC and progenitor cell differentiation exhibit advantages such as faster differentiation speed, higher efficiency, and specific induction of catecholaminergic dopaminergic neurons, which is particularly important for treating Parkinson's disease. Additionally, CAP treatment is controllable, allowing precise modulation of its biomedical effects by adjusting plasma source parameters. In summary, CAP-induced neural differentiation is realized via activation of the Trk/Ras/ERK signaling pathway, offering new prospects for treating neurodegenerative diseases.

CAP can also be combined with other technologies to treat the brain. Recently, Menéndez-Coto investigated the effects of a device that combines CAP therapy with efficient elimination of environmental pollutant nanoparticles, on brain activity in aged mice [265]. The findings indicated that this dual approach not only facilitated the removal of nanoparticles from the environment but also promoted a barrier-free laminar flow of anions. This flow could penetrate the skin and mucous membranes, directly influencing individuals. Consequently, it enhanced energy capacity, which is vital for mitigating neurodegenerative processes, and alleviated endoplasmic reticulum stress. Furthermore, the treatment activated autophagic clearance, reducing cerebral protein aggregates. These aggregates, toxic accumulations frequently associated with age-related neuronal diseases, were minimized. Consequently, key markers of neurodegenerative pathology,

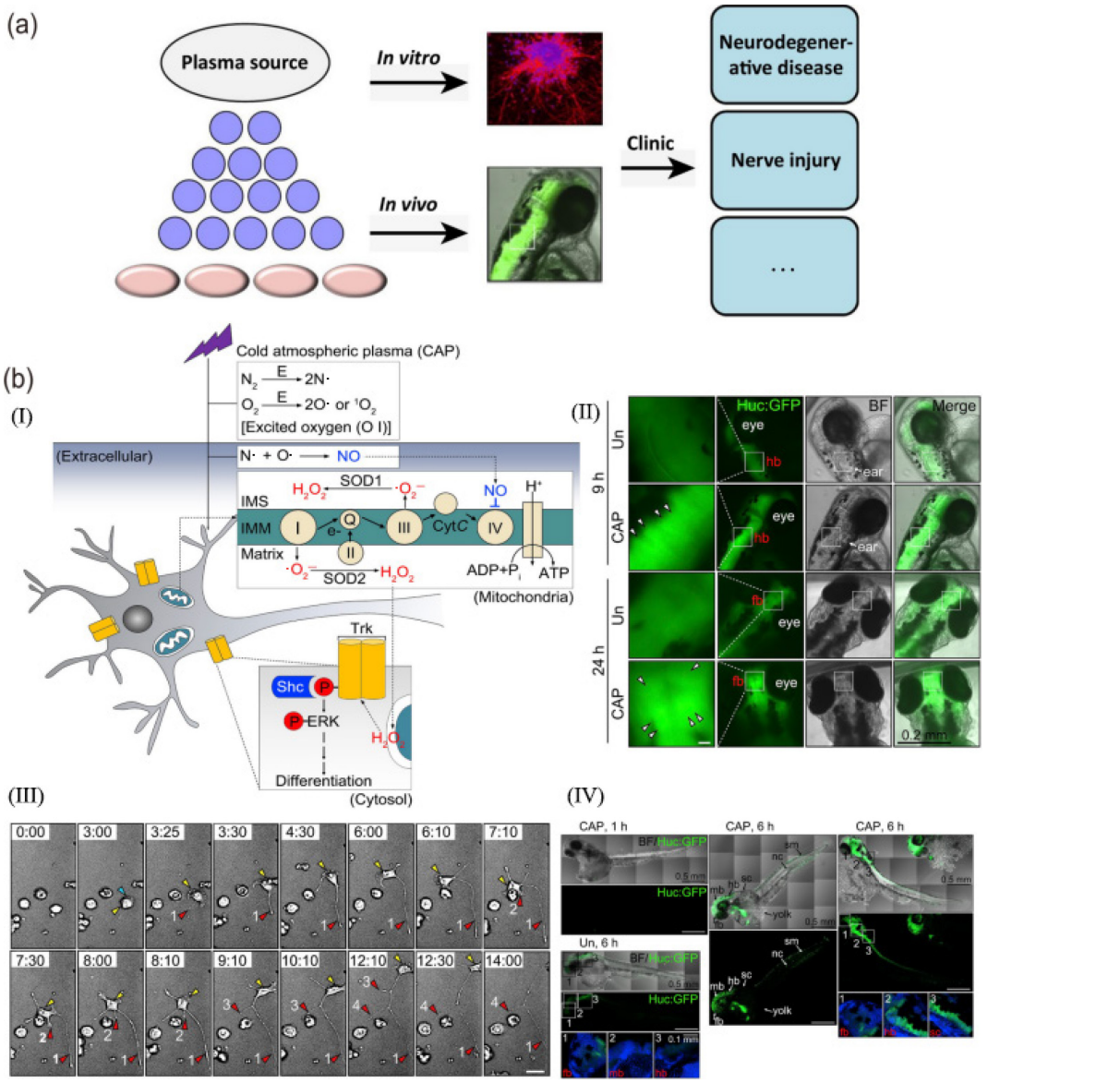

Figure 12 CAP for neurological disease treatment. (a) Promising CAP treatment for neurological disease. CAPs can accelerate and selectively direct the differentiation of nerve cells into neurons *in vitro* and *in vivo*, showing potential for future clinical treatment of neurological diseases [264]. Reproduced with permission from Ref. [264], © Elsevier Ltd. All rights reserved 2018. (b)(I) A working model of CAP-induced neural differentiation; (II) functionally specified mature neurons differentiate from NPCs driven by CAP treatment; (III) neural differentiation of zebrafish driven by CAP treatment during late embryogenesis; (IV) *in vivo* verification of CAP-induced neural maturation in zebrafish [62]. Reproduced with permission from Ref. [62], © Elsevier Ltd. All rights reserved 2017.

including hyperphosphorylation of tau protein and β-amyloid deposition, were diminished, demonstrating the device's efficacy at the brain level.

4.5 CAP for other diseases treatment

Inhaled NO, a selective pulmonary vasodilator, has been proven effective for pulmonary hypertension treatment across adult and pediatric populations. However, it is also one of the most expensive medications in neonatal medicine. To devise a lightweight and economical device for bedside treatment or portable application, Yu et al. generated NO from air or gas mixtures comprising 90% O_2 and 10% N_2 using electrical plasma based on pulsed discharge technology (Fig. 13(a)). Two distinct NO generation systems were developed and evaluated: an offline NO generator and an online NO generator integrated directly into the inspiratory line. Both generators were capable of producing therapeutically required concentrations of NO (5 to 80 ppm) at gas flow rates ranging from 0.5 to 5 L/min (Fig. 13(a)(II)). Calcium hydroxide scavengers were used in both generators to eliminate potential toxic gases like NO_2 and O_3 produced in the plasma. In a lamb model of acute pulmonary hypertension, plasma generated NO exhibited equivalent efficacy to NO supplied from cylinders in promoting rapid pulmonary vasodilation, reducing elevated pulmonary arterial pressure (PAP), and lowering pulmonary vascular resistance index (PVRI) (Figs. 13(a)(III) and 13(a)(IV)). This study suggests that future lightweight and portable electrical plasma NO generators have the potential to explore the application of NO inhalation therapy in outpatient settings for patients with chronic pulmonary hypertension and chronic obstructive pulmonary disease [81].

Ischemic heart disease, a complex chronic condition

characterized by pathological alterations in coronary arteries and myocardium, encompasses multiple stages and diverse clinical manifestations [266]. It stands as the leading cause of cardiovascular-related deaths and chronic heart failure globally. In the treatment of ischemic heart disease, abnormal sulfide catabolism, particularly the accumulation of hydrogen sulfide, inhibits mitochondrial respiratory function, thereby exacerbating clinical outcomes post-ischemic injury. However, effective targeted therapies remain scarce.

Recent research has revealed that supersulfides, generated through the catenation of sulfur atoms, are vital to intracellular electron transfer processes and may serve as a promising chemical entity for treating ischemic heart disease. Nishimura et al. reported an innovative study utilizing CAP irradiation of cysteine (Cys) to effectively protect mouse hearts from I/R injury and rat cardiomyocytes from oxygen-glucose deprivation/reoxygenation (OGD/R) injury by inhibiting the supersulfide metabolism [68]. The mechanism involves plasma irradiation of thiol-containing amino acid Cys, yielding supersulfides such as cysteine persulfide (CysSSH). When applied to cardiomyocytes, these supersulfides prevent mitochondrial dysfunction by maintaining mitochondrial bioenergetics. Additionally, plasma-irradiated Cys products promote sulfide catabolism, further safeguarding mitochondrial function. Compared to hydrogen sulfide and other sulfide species, plasma-synthesized supersulfide minimizes sulfide toxicity, offering a new approach for treating ischemia-reperfusion injury (Fig. 13(b)). Furthermore, the study demonstrated enhanced cellular protection through upregulation of sulfide-quinone oxidoreductase (SQOR) expression. Local administration of Cys-treated mouse hearts resulted in significantly reduced infarct size and maintained

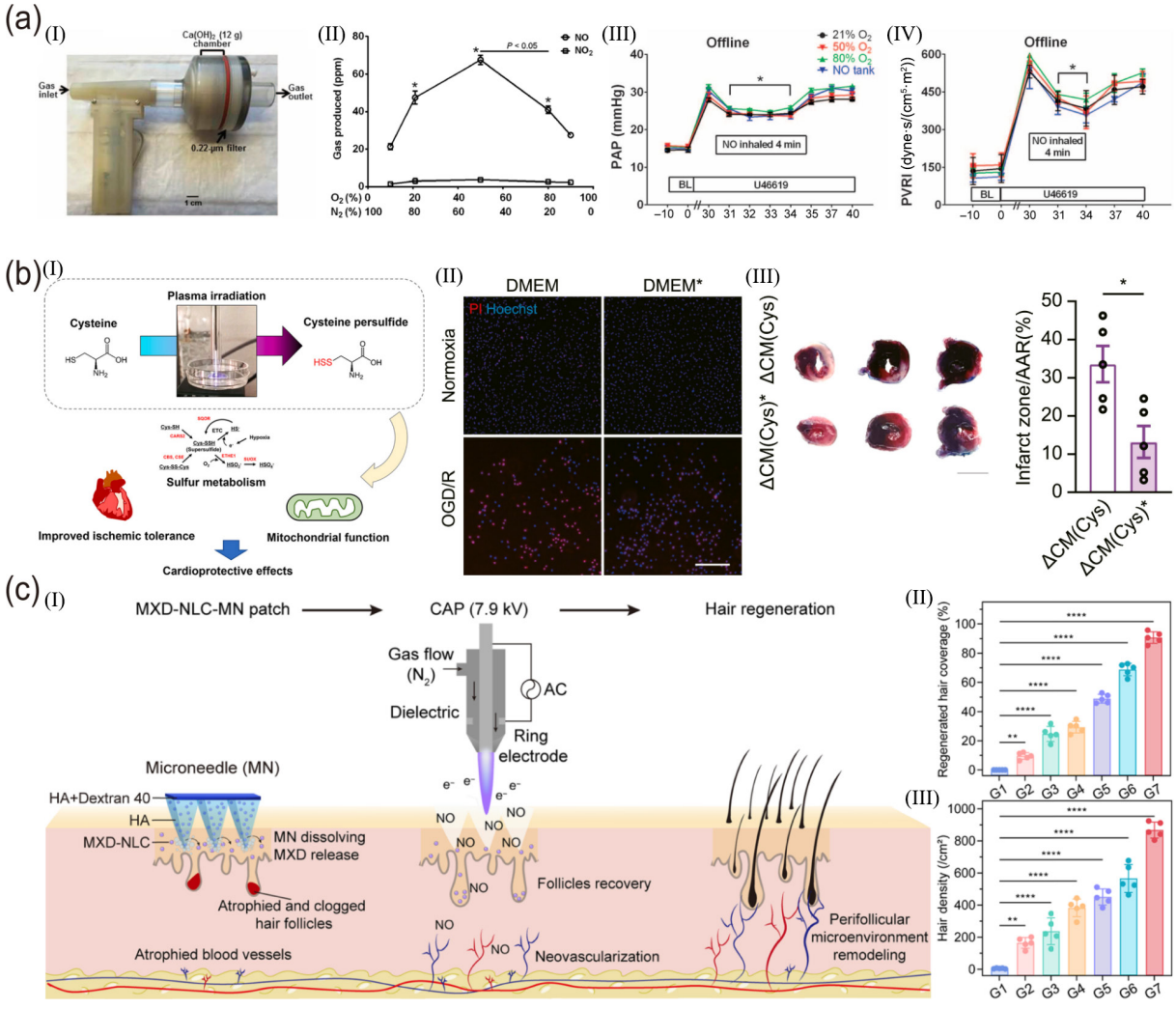

Figure 13 CAP for other diseases treatment. (a) Generating NO via pulsed electrical discharge in air for portable inhalation therapy: (I) schematic of electric plasma NO generators; (II) NO and NO_2 levels are produced by an inline electric plasma generator; breathing electrically generated NO (offline continuous generation) reverses pulmonary vasoconstriction in lambs: (III) PAP and (IV) PVRI before and after inhaling 40 ppm NO [81]. Reproduced with permission from Ref. [81], © American Association for the Advancement of Science 2015. (b) CAP-irradiated cysteine protects cardiac ischemia/reperfusion injury: (I) schematic diagram of the protection of cardiac ischemia/reperfusion injury by CAP irradiation of cysteine through preservation of supersulfides; (II) effect of plasma-irradiated DMEM (DMEM*) on cardiomyocyte injury under normoxia or oxygen glucose deprivation/reoxygenation (OGD/R) condition; (III) cardioprotective effect of plasma-irradiated Cys products in ischemia/reperfusion mice model, infarcted zone/area at risk (AAR) ratio following I/R [68]. Reproduced with permission from Ref. [68], © Nishimura, A. et al. Published by Elsevier B.V. 2024. (c)(I) Diagram of a multimodal therapeutic platform integrating MXD-NLC-MNs and CAP to reshape the perifollicular microenvironment for androgenetic alopecia therapy; (II) hair coverage in different treatment groups as indicated in the panel on day 28 post-depilation; (III) hair density in different treatment groups as indicated in the panel on day 28 post-depilation [80]. Reproduced with permission from Ref. [80], © Tsinghua University Press 2024.

supersulfide levels post-I/R (Fig. 13(b)(III)). Although the chemical supersulfide activity generated by plasma irradiation of Cys is estimated at only ~ 80 nM of inorganic sulfur Na_2S_4, its cardioprotective effects are more sustained. Additionally, the study found that SQOR is crucial for maintaining supersulfide metabolism and contributes to cardioprotection against I/R injury induced by plasma-irradiated Cys, offering new insights into potential therapies for ischemic heart disease.

Moreover, previous studies have indicated that the transient microchannels formed by microneedle patches in the skin can promote the transdermal delivery of CAP-generated NO to the affected areas of the hair follicles (Fig. 13(c)). On this basis, the combination of mechanical action of microneedles and the therapeutic effects of minoxidil worked synergistically in treating androgenetic alopecia [80].

5 Biosafety of CAP

In biomedical applications, CAP exerts its therapeutic effects primarily through RONS. However, excessive RONS may disrupt cellular redox homeostasis, inducing cytotoxicity such as DNA damage and mitochondrial dysfunction [267, 268]. This raises concerns about the mutagenic risks of plasma therapy. Yet accumulating evidence indicates that such DNA damage is transient and effectively repaired within 24 h [269]. Even at doses 5–10 times higher than clinical recommendations, CAP exhibited no mutagenic risk [270]. Consistently, CAP showed no genotoxicity to human cells *in vitro*, and repeated subcutaneous injections of PASS in rats induced neither DNA damage nor carcinogenic changes in skin or major organs [267, 271]. These findings are corroborated by numerous clinical studies in dermatology, which have consistently reported good tolerability and no significant side effects [272, 273].

However, mammalian hypoxanthine-guanine phosphoribosyl transferase assays have shown that prolonged plasma exposure at the cellular level can induce cytotoxic effects and increase mutagenicity potential [274]. Clinically, plasma applications remain short-term, and long-term follow-up data are still limited [272]. Notably, like other physical or chemical agents, CAP exhibits dose-dependent effects [275]. By adjusting exposure time and reactive species yields, safe and effective therapy can be achieved without toxicity. However, the lack of a standardized definition of plasma "dose" complicates the comparison of biological effects across devices and application scenarios, hindering device selection and parameter optimization. Establishing a safe dose limit is therefore essential for patient safety, which in turn necessitates accurate detection and quantification of CAP-generated reactive species.

6 Future perspectives

The field of plasma medicine has witnessed significant progress in recent years. This review provides a systematic academic overview of the promising prospects of CAP in the treatment of chronic diseases, with a particular focus on its distinctive therapeutic advantages and ongoing research efforts directed at addressing current challenges. As fundamental research and clinical demands continue to reinforce each other, the translational development of plasma technology is accelerating, and its integration into clinical practice is becoming increasingly robust (Fig. 14). Current research on CAP indicates that it primarily relies on generated RONS to induce a range of significant biological effects, which exhibit concentration-dependent characteristics. Thus, by modulating the concentration of RONS generated by CAP, it may be possible to elicit biological effects on demand. For instance, high-intensity CAP can achieve sterilization and tumor ablation, whereas low-intensity CAP can promote cell proliferation and tissue regeneration. Although plasma devices and delivery methods have been improved, and plasma therapy has been clinically applied in several countries with demonstrated efficacy and accessibility, the field remains in its infancy. Many scientific and/or technical issues still need to be resolved to achieve the next leap in plasma therapy development:

(1) The dosimetry of plasma is a critical issue that remains unresolved. Current methods can only marginally detect the active components and physical properties within the plasma. Considering the complexity of plasma reactions, there is a deficiency in highly specific quantification methods to accurately measure its active substances, particularly short-lived reactive molecules, which are crucial for direct clinical applications.

(2) The diversity of plasma devices and the lack of standardization complicate cross-device comparisons and setting adjustments [21]. There is no consensus regarding treatment frequency, dosage, or optimal timing, necessitating further research to clarify the influence of various treatment parameters.

(3) The efficacy and safety profile of CAP in treating deep-seated lesions are still unclear, and long-term follow-up data are lacking because of the short clinical application period [272]. Potential adverse reactions, sensitivities, and risks of cancer or precancerous conditions require further assessment. Notably, research on plasma therapy for chronic diseases and autoimmune disorders is still in its primitive stage, and the capacity for long-term and frequent treatment of deep tissues remains to be determined. Although plasma technology shows innovative potential in wound care, therapy, and autoimmune disease management, especially when combined with effective delivery methods, its application in home treatment is limited by device conditions.

(4) Plasma medicine research has mainly focused on chemical effects, with the physical effects of plasma, particularly cell death mechanisms induced by physical factors, being underexplored. The potential for non-invasive cancer treatment warrants further investigation. Moreover, CAP-induced transfection, a novel physical transfection method characterized by its simplicity, painlessness, effectiveness, and non-contact nature with minimal impact on cell viability and physiological functions, holds promise for providing new avenues in chronic disease treatment [276–278]. However, this technology is still in the exploratory stage and requires ongoing development and refinement.

In the treatment of chronic diseases with plasma, future research should focus on addressing core issues such as dosimetry/safety assessment and accessibility in order to promote its wider application in medicine. (1) Addressing the vast existing data on plasma therapy outcomes, artificial intelligence is employed for in-depth processing and comprehensive analysis, aiming to devise appropriate plasma treatment protocols. This approach holds promise in addressing the challenges posed by the variety of plasma devices and the lack of unified standards, potentially emerging as a viable research direction. (2) To achieve a more effective application of plasma for chronic disease treatment, it is essential to continuously conduct basic research, advance the miniaturization and cost reduction of plasma devices to facilitate their being widely accessible in communities and households, and integrate digital

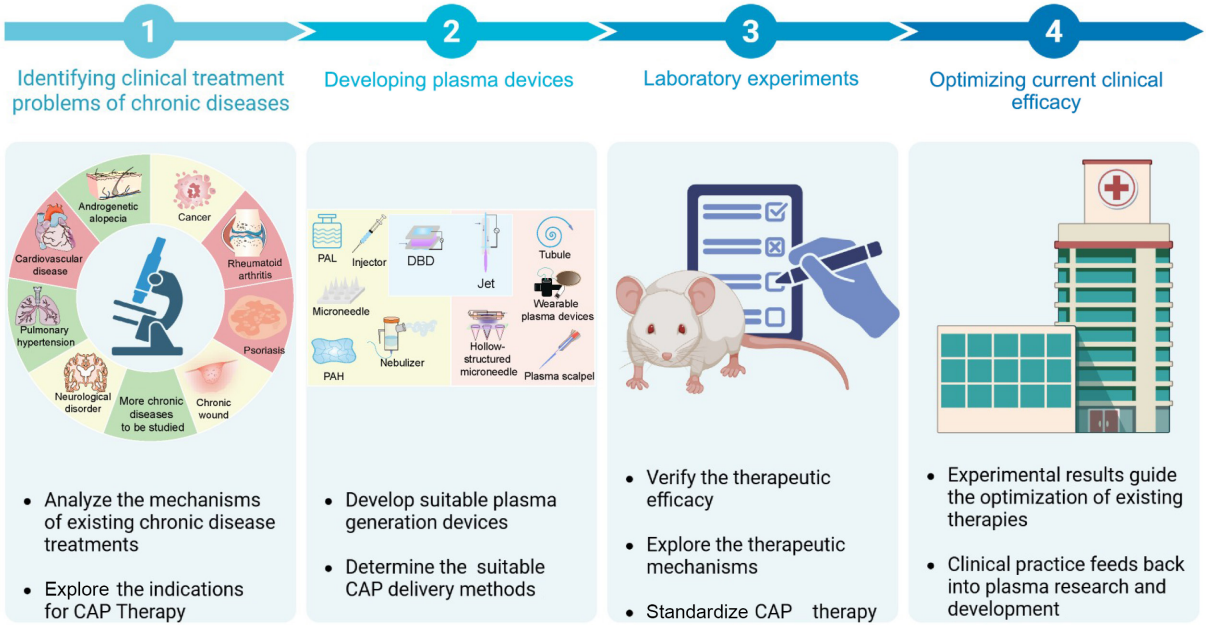

Figure 14 A roadmap for realizing the wider and more effective application of plasma in clinical disease treatment. Summary of the key steps for the clinical translation of plasma as well as the solutions proposed in this manuscript. Partially created with BioRender.

technology to achieve intelligent remote monitoring for chronic disease management. This will enable clinicians to remotely monitor patients' conditions and allow patients to self-administer treatment at home using plasma devices, which is particularly crucial for chronic diseases requiring long-term self-management. Consequently, the refinement of plasma devices and delivery methods that meet clinical application needs is essential. (3) Lastly, given that chronic disease patients often suffer from multiple comorbidities [279, 280], it is of significant importance to investigate whether CAP can reduce the intake of multiple therapeutic drugs simultaneously, optimize dosing for combined CAP and pharmacological therapies, and study their synergistic effects and treatment mechanisms for realizing the clinical application of CAP as an adjuvant therapy [238, 267].

7 Conclusions

In conclusion, CAP exhibits extensive potential for clinical application in modern medical practice, and no drug resistance associated with CAP treatment has been observed to date. Due to its excellent selectivity, it does not produce marked adverse effects on healthy cells. These characteristics enable the long-term application of plasma for chronic disease treatment, thereby reducing drug usage. This approach alleviates the economic burden and side effects related to conventional chronic disease treatments. However, it must be acknowledged that there is currently a lack of standardized plasma treatment protocols and large-scale clinical trials. The convenient delivery to certain lesion sites and the dosage of active components are difficult to ascertain. These issues must be overcome for plasma application in chronic disease therapy. Based on the premise that chronic progressive diseases are viewed as a collection of symptoms, their treatment is often downgraded to symptom management [281]. With in-depth research into the CAP mechanisms in disease treatment, the capacity of reactive species produced by CAP to target multiple biomolecular targets within organisms suggests that CAP holds great potential for chronic disease treatment [282].

Data availability

Not applicable.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution statement

T. J. H.: Writing – review & editing, writing – original draft, investigation, conceptualization. G. A. M.: Writing – review & editing, investigation. C. X. L.: Investigation. R. Z.: Writing – review & editing. Z. W. C.: Writing – review & editing, conceptualization. Z. Z. W.: Writing – review & editing, writing – original draft, conceptualization. Z. T. C.: Writing – review & editing, writing – original draft, funding acquisition, conceptualization.

Informed consent

Not applicable.

Ethics statement

Not applicable.

Use of AI statement

No AI tools were used in the research design, data collection, analysis, or interpretation. During the preparation of this work, the authors used AI tools (DeepSeek, ERNIE Bot, and Doubao) for language refinement. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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