Cold atmospheric plasma applications in dermatology: a systematic review

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Abstract

Cold atmospheric plasma (CAP) applications can potentially lead to effective therapy for numerous skin diseases. Our aim is to systematically review the available data and map the use of CAP in dermatology. PubMed, Embase and Web of science were explored before 2020 for studies regarding the use of CAP in dermatology. A total of 166 studies were finally included. 74.1% of these studies used indirect CAP sources. Most studies used plasma jet (67.5%). Argon was the mostly used working gas (48.2%). Plasma application itself could be direct (89.2%) and indirect (16.3%). The proportion of studies with in vivo results remained 57.2%, of which most concerned direct plasma treatment (97.9%). Analyses performed indicate that CAP has been beneficial in many skin disorders. While, most CAP applications were focused on wound healing and melanoma treatment. This study provides a brief overview of CAP sources and relative medical applications in dermatology.

Keywords: Cold atmospheric plasma; Therapeutics; Dermatology; Skin diseases

1. Introduction

Plasma is an ionized gas that is defined as the fourth physical state of matter[1, 2]. Developments in cold atmospheric plasma (CAP) science and engineering have promoted new understanding and application of plasma in biomedicine. With temperatures close to that of vertebrate systems, CAP has been actively studied for a wide range of medical applications, which has given rise to an innovative and emerging field, referred to as plasma medicine [2-5]. Various technologies have been used to design new sources of CAP. At present, CAP for medical applications is generally generated by two major types of devices: indirect and direct plasma sources[6, 7]. Indirect plasma is produced remotely, and the plasma components are delivered to the biological target via carrier gas. While direct plasma is ignited in the gap between an isolated (dielectric) high-voltage electrode and the surface to be treated, which means that the treated biological sample or living tissue serves as the counter electrode necessary for plasma ignition. Its defining feature is the presence of dielectric material between the electrodes[6]. Based these principles above, two CAP devices, the plasma jet and the dielectric barrier discharge (DBD), have been developed and widely used in plasma medicine. CAP has been employed for various medical fields, such as oncology, otolaryngology, gastroenterology and odontology. Moreover, various indications have also been proposed, such as infections, chronic wounds, blood coagulation and malignant diseases[2, 8, 9].

Skin disease is one of the most common human illnesses and causes a huge burden in the global context of health. It affects between 30% and 70% of individuals, with even higher rates in at-risk subpopulations[10]. As skin is the outmost organ of the human body and most easily accessible, CAP therapy is easy to implement, allowing the clinical application of CAP in dermatology to have promising possibilities. Recently, the use of CAP has gained wide attention in the field of dermatology and also gained promising results[8, 11]. Reports have shown that CAP could be effective in numerous skin diseases. Although the majority of published studies reported results obtained from laboratory-based experimental work, a number of clinical trials involving patients treated with CAP for skin diseases had also been reported[12-15]. Cold plasma comprises a complex mixture of biologically active components such as charged particles, electric current, UV radiation and reactive gas species which can act synergistically[4, 16]. It is generally accepted that reactive oxygen and nitrogen species (RONS) are the major agents responsible for plasma-promoted biological effects. CAP is a potent source of a multitude of gaseous RONS. The latter is subsequently transported to cells and tissues where RONS lead to redox-based changes in lipid and protein structures, stimulation of redox-controlled cell pathways and finally elicit biological responses[17]. Furthermore, recent studies suggest that CAP has the potential to enhance the percutaneous absorption of transdermal drugs, and claim the important role of CAP with nanotechnology in cancer treatments[18, 19]. The aim of this systematic review is to screen existing articles that have studied the use of CAP in the treatment of skin diseases and provide an overview of this constantly evolving and promising field of plasma use in dermatology.

2. Materials and methods

2.1 Search strategy and study selection

This systematic review was performed in accordance with the PRISMA (preferred reporting items for systematic reviews and meta-analyses) guidelines. We did a systematic search on

articles published before 2020, in the databases PubMed, Embase and Web of Science. We developed a search strategy and adapted it for each database using a combination of Medical Subject Headings (MeSH) and free texts including keywords related to cold atmospheric plasma and medical field (dermatology). Figure 1 showed the flowchart of the study selection process.

Duplicate studies were excluded. All potentially relevant studies, identified through the search strategy, were screened based on titles and abstracts. Original reports regarding the use of CAP as a treatment therapy in dermatology were included. Full texts of the selected records were obtained for definitive inclusion. Reviews and articles without full text electronically available were excluded. Studies regarding argon plasma coagulation (APC) and plasma-based devices in cosmetics were also excluded because those devices rely on thermal plasma effects[20, 21]. The bactericidal effects of CAP on different kinds of pathogens have been extensively studied. Studies were also excluded if they exclusively reported the bactericidal effects.

2.2 Data extraction

The following data were collected: the country of author's affiliation, the type of methodology (in vitro, ex vivo, in vivo), the type of plasma device used (direct, indirect plasma source), the process gas (argon, helium, oxygen, nitrogen, air, mixtures thereof), the treatment regimen (direct, indirect treatment), and the cell type treated (origin of the cell line). The authors of an article may come from more than one country. Consequently, a study may be related to several countries. In addition, some studies may involve more than one kind of skin disease, methodology or treatment regimen. A standardized extraction form was created to collect data according to the outcomes detailed above (Table S1).

3. Results

An initial search identified 2159 studies. After removal of duplicates and initial screening, we reviewed 158 papers. Eight records were founded by hand searches. Finally, 166 articles were included in the systematic review. From 2007, the number of original articles on the therapeutic effects of skin diseases with CAP has grown overall (Figure 2(a)). There were 56 studies from Germany, 31 studies from USA, 21 studies form China, 20 studies from Korea and 15 studies from Japan (Figure 2(b-c)). No systematic review focusing on this topic was present in our search.

3.1 Plasma production

During recent years, a broad spectrum of different plasma sources (called by many different names and abbreviations) have been designed and dedicated for biomedical applications. The majority of these studies used indirect CAP sources (123, 74.1%) (Figure 3(a)). Mainly two major categories of CAP devices, the plasma jet and the DBD, have been tested and widely applied for medical purposes[22]. Among all the studies we included, 112 studies used the plasma jet device, and 46 studies used the DBD device. Besides, three studies did not specify the type of plasma production. CAP can be produced by discharges in noble gases, air or other mixtures. We found that argon was the most-used working gas (48.2%), followed by air (25.9%) and helium (24.1%) (Figure 3(b)). Until now, several kinds of plasm devices have received certification as a medical device. Among these, KINPen MED (Germany), SteriPlas (AdTec Ltd., Japan) also known as MicroPlaSter, and PlasmaDerm (Cinogy GmbH, Germany)

were principally utilized in dermatology (Figure 3(e)). The three famous products for skin wound healing based on plasma jet, FE-DBD and SMD, respectively.

3.2 Plasma application

CAP treatment can act in two operation modes representing direct and indirect plasma treatment, respectively. Direct treatment applies plasma plumes directly on cells or living tissues. Cells, in direct plasma application in vitro, are always covered by a layer of culture medium or suspended in the medium to avoid the "drying effects" of the plasma on the cells [23-25]. Animal models and human tissues are directly exposed to the discharges in vivo. In contrast, the medium or solution irradiated or activated by plasma is used in indirect plasma application. This is an indirect plasma treatment method in which plasma activated media (PAM) is added to the culture supernatant of separately cultured cells or injected into tested subjects without direct plasma irradiation[26, 27]. Indirect treatment uses PAM, which demonstrates similar effectiveness to direct treatment, with the advantage that PAM can be injected into the body and therefore can reach tissues inaccessible through direct irradiation. Figure 3(f) highlighted the predominant use of direct plasma treatment (148, 89.2%) rather than indirect treatment (27, 16.3%). However, in recent years, the number of studies using indirect plasma treatment has increased, importantly. All these investigations on dermatologic plasma applications were based on numerous in vitro, ex vivo and in vivo experiments. It revealed that 95 studies contained in vivo results, comprising ex vivo tissue model (14, 8.4%), animal (55, 32.7%) and clinical studies (36, 21.7%) (Figure 4(a-b)). The proportion of studies with in vitro results remained 49.4% (82 studies). Both human (46, 27.7%) and murine (32, 19.3%) cell lines had been used to elucidate the therapeutic effect of CAP. In vitro experiments based on human cell lines accounted for 59.0%, and melanoma cell accounted for 33.3% particularly. Murine cell lines were mostly used in the melanoma researches (87.5%). Seven clinical trials have been identified through the International Clinical Trials Registry Platform and ClinicalTrials and four of these are ongoing.

3.3 Type of skin diseases

Up to now, the main field of plasma medicine is dermatology. The potential dermatological plasma applications were presented in Figure 4(c). Proof of the therapeutic effectivity of CAP was based on summarizing data as well as presented case reports. The most commonly reported was wound healing (59, 35.5%), followed by melanoma (54, 32.5%). To provide an overview of the current state of knowledge on the safety of plasma for biological applications, in vitro cytotoxic studies along with in vivo findings from animal and human studies were reviewed. Of those included studies, about 6.6% involved safety assessment. As plasma can penetrate nail plate, fungal infections like onychomycosis could be improved or healed by CAP application. Besides, squamous cell carcinoma, psoriasis, atopic dermatitis, improving skin microcirculation and skin permeability as well as modulating melanogenesis were in the top 10 most commonly studied dermatological applications. In vivo efficacy of CAP in treating pruritus, actinic keratosis, rosacea, warts, hailey-haileydisease and herpes zoster have also been reported.

4. Discussion

CAP has been gaining increasing interest as a new treatment modality in the field of dermatology. This is reflected by the constant increase in the data about the use of CAP

devices in the treatment of skin diseases. So far, a broad spectrum of different plasma sources with various names dedicated for biomedical applications have been reported[2]. While, research activities were mainly focused on the plasma jet and the dielectric barrier discharge (DBD). Summarizing the results demonstrated that the plasma jet was the predominant discharge device used for plasma production. Plasma can be generated by discharges in the air, noble gases, or desired mixture. We showed that argon was the most-used working gas. Studies show that plasma application itself can be direct and indirect. While, direct CAP treatment was the most represented, but indirect treatment appeared to be increasing in recent years. Indirect treatment had demonstrated similar effectiveness to direct treatment with the advantage that indirect treatment using plasma activated media (PAM) made CAP treatment more like a drug therapy[28].

CAP has complex effects on cells and tissues, and these effects are studied extensively for medical use. The major biologically active component of plasma is the variety of RONS, which may act directly as signaling molecules or via inducing oxidative stress related triggering of various pathways or by other mechanisms, such as changing intracellular ion concentrations. CAP treatment can induce formation of numerous reactive species in the biological targets, inducing a stimulating effect on cell proliferation or apoptosis dose-dependently. We reviewed the evidence of the clinical benefits of plasma applications in dermatology. It found that most researches and medical applications were related to wound healing and melanoma treatment. The initial intention to use CAP for medical purpose was first of all based on its use for microorganism inactivation. Therefore, in the field of dermatology, the main focus of plasma medical applications was treatment of infectious skin diseases. The bactericidal effect of CAP on different kinds of pathogens had been extensively studied, and its antimicrobial effect is undisputed now. Two crucial scenarios of the plasma-induced bacterial death have been proposed: physical destruction and death-leading biochemical reactions[29]. Studies have also shown that CAP is able to destroy biofilms, which are dense and complex microbial communities and known to prevent disease eradication by conventional antimicrobial therapy[30]. All this led to the hypothesis that plasma might be an alternative solution for antiseptic treatment of chronic infected wounds. Whereas, there are several aspects of CAP that may contribute to an improved wound healing. Besides the proven antimicrobial efficacy, CAP treatment can modulate wound healing by directly influencing the biological behavior of epidermal and dermal cells[31, 32]. A huge number of in vitro studies have demonstrated CAP-triggered wound healing based on the stimulation of cell proliferation, extracellular matrix (ECM) protein synthesis, changes of junctional proteins and cytoskeletal architecture[33, 34]. CAP treatment can promote keratinocyte and fibroblast proliferation and migration, and induce expression of genes relevant to wound healing, such as type I collagen, transforming growth factors (TGFβ1/2), and alpha-smooth muscle actin (α-SMA)[8]. Plasma-mediated RONS and fibroblast growth factor-2 (FGF2) release from fibroblasts can promote endotheliocyte migration, proliferation, and tube formation. Studies have showed that plasma treatment triggers a coordinated action of redox-sensitive transcription factors known to be important in wound healing. The nuclear factor erythroid 2-related factor 2 (Nrf2) is a crucial translator for redox signaling. Plasma-generated reactive species are likely translated via the redox-sensitive Nrf2 signaling and significantly alter the Nrf2 pathway[12]. Plasma-induced regulation of Nrf2 and its down-stream targets can promote

re-epithelialization, angiogenesis, oxidation of lipid layer and TGFβ signaling, ultimately reinitializing the sequence of events necessary for healing. Beyond that, cutaneous oxygen saturation and microcirculation can be enhanced after plasma treatment, which in turn improve vascularization of the wound site. Also, wounds, treated with CAP, closed significantly faster in in vitro wound model (scratch assay) and experimental animals. Clinical trials and case reports also revealed beneficial effects of plasma therapy on acute and chronic wounds, like diabetic foot wounds and pressure ulcers[32, 35-37]. Important in vivo studies on the efficacy of CAP in wound treatment as well as reviews were published very lately in 2020, which also nicely corroborate the results of our systematic review[38-40]. The effect of CAP on tissue regeneration has also been elucidated for its potential in scar treatment. Interestingly, CAP is able to stimulate physiological collagen genesis while inhibiting the pathological synthesis of the protein. In vitro and in vivo studies have shown this selective effect in dermo-aesthetic disorders such as scars and keloids[41].

CAP has also been proposed as a potential new anti-cancer therapy. Investigations with different cancer cell lines and CAP devices showed that CAP suppressed cell migration and invasion and induced apoptosis with high selectivity[42]. Plasma treatment can cause growth inhibition, DNA damage and cell cycle arrest by stimulating the increase of intracellular RONS, and then induce apoptotic cell death of malignant proliferative cancer cells. Eliminating these active species could effectively inhibit the occurrence of cell apoptosis. Many signal transduction pathways including the TNF/ASK1, Ras/MAPK, and ATM/p53 signaling pathways are affected by CAP and associated with the anticancer effect[43, 44]. Plasma treatment can also significantly increase the number of calcium ions (Ca^{2+}) and thus induce the endoplasmic reticulum (ER) stress mediated apoptosis pathway[45]. Studies have reported plasma-triggered mitochondria dysfunction activating caspase-independent and caspase-dependent apoptosis signal pathways. CAP has been shown to induce proapoptotic effects more efficiently in cancer cells than that of normal cells. Various hypotheses to explain the selective killing have been proposed. Cancer cells are characterized by a more active metabolic status, resulting in higher basal ROS levels and making these cells more susceptible to RONS generated by plasma. In addition, an increase in aquaporins (AQPs) and a decrease of cholesterol in the plasma membrane of cancer cells would facilitate RONS transmembrane diffusion. It is possible that the combination of these factors above favors the selective elimination of cancer cells by plasma. Many cancers may benefit from the use of CAP treatment, and the most predominant field of the study is melanoma, one of the most aggressive skin cancer diseases. Publications have revealed the therapeutic effects of CAP on melanoma both in vitro and in vivo. In vitro studies showed growth inhibition of melanoma cells in culture. Murine melanoma cancer cell lines and human melanoma cell lines represented 16.9% and 15.7% of the studies, respectively. In vivo interventions were mostly performed on subcutaneous tumor xenografts in mice. Decreased tumor growth or a reduction of tumor volume in mouse models has been observed after CAP treatment. The immune system plays an important role in cancer treatment. CAP has been found to increase the release of the danger-associated molecular patterns (DAMPs) and activate the immune system[46]. In addition to localized effects, CAP has been reported to induce immunogenic cell death (ICD) in melanoma cells, and elicit specific systemic antitumor immune responses[23, 47-49]. Therefore, CAP treatment on accessible lesions might support immunotherapy that has

become an important treatment option in metastatic melanoma. Dermatological applications of CAP in skin cancers also including squamous cell carcinoma and precancerous conditions, like actinic keratosis[50, 51].

Antifungal treatment is another scope for plasma treatment in dermatology since the excellent killing of the most clinically relevant fungal strains by CAP has been shown in vitro. A significant reduction of fungal targets after plasma treatment has also been shown on fungal infection model nails and toenails [52-54]. Taken together, these studies indicate a promising application of CAP as an innovative therapy for onychomycosis. Furthermore, dermatologic diseases with parasitic involvement like demodicosis could benefit from plasma treatment[55]. Improved acne symptoms have been demonstrated after using CAP[56]. Recently, many other skin diseases also came into focus for treatment with CAP, such as pruritus, atopic eczema, psoriasis and herpes zoster[57-60].

5. Conclusion

CAP has gained increasing amounts of attention, and studies in plasma medicine increase with pace. Due to the anatomical position, skin presents an ideal organ for CAP therapy as it is easily accessible. Plasma application in dermatology is now one of the most studied fields in plasma medicine. Here, we systematically reviewed the available literature on the potential dermatological applications of CAP. Results of the studies that were summarized within this review showed that CAP may hold a good promise as a new treatment modality for a variety of skin disorders. Its antimicrobial, anti-itch, pro-apoptotic, and anti-inflammatory effects were already demonstrated in in vivo and in vitro experiments. So far, successful observations have been made for the treatment of different types of wounds, melanoma, psoriasis, atopic eczema, pruritus, actinic keratosis, warts, and so on. Moreover, plasma treatment can act in concert with other drugs and optimizing percutaneous drug delivery. Meanwhile, a good compatibility of plasma treatment on skin has been reported. Although CAP applications in dermatology are on their way to clinical reality, the precise molecular mechanisms underlying these effects still require further research. Continuing efforts will be necessary to further explore the full therapeutic potential of CAP and to fully understand its mechanisms of action, thus providing increasing evidence for the use of CAP as a treatment option for dermatological diseases.

Conflicts of interest

The authors have no conflicts of interest to declare.

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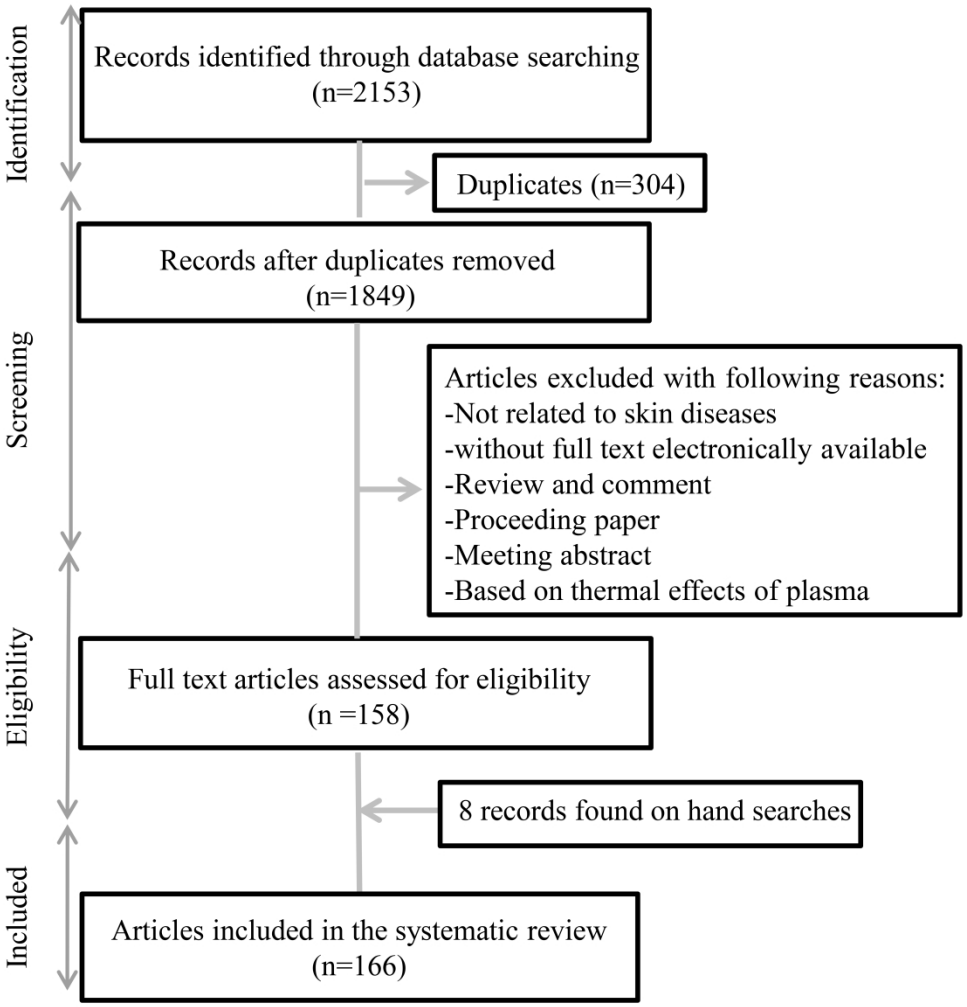
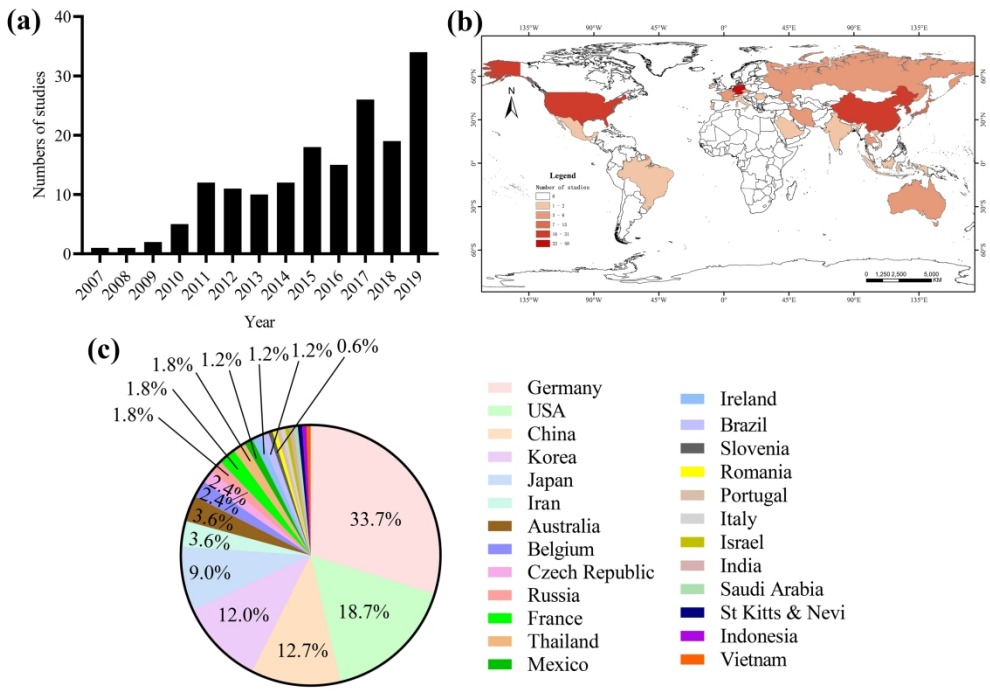
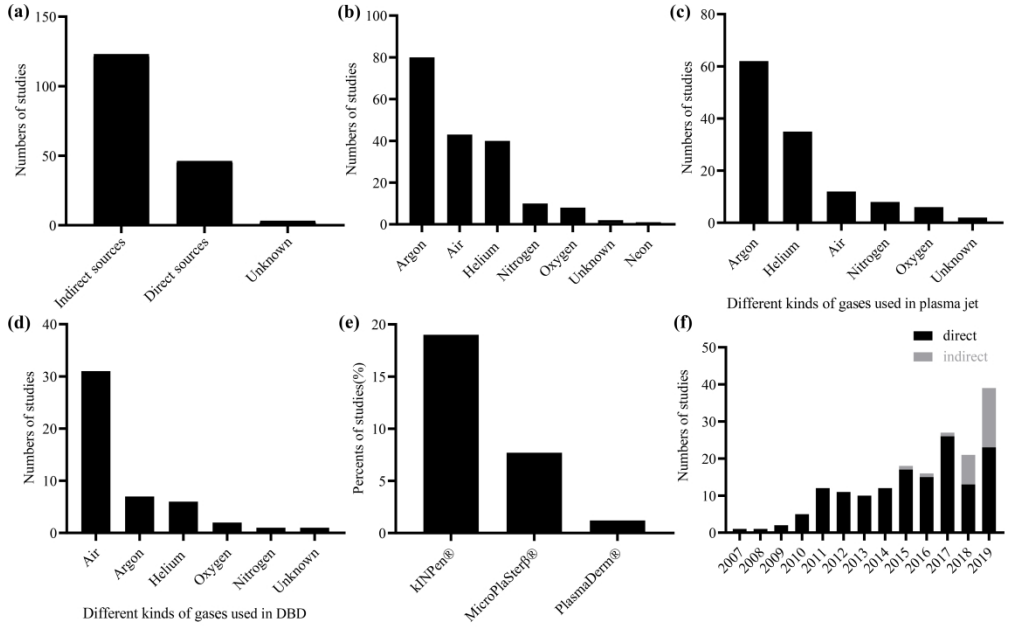
Cold atmospheric plasma (CAP), known for its innovative medical applications, has gained wide attention in the field of dermatology. Studies regarding the clinical benefits of plasma applications in dermatology and the methods adopted were systematically reviewed here, providing an overview of the current state of knowledge on CAP for potential dermatological applications.



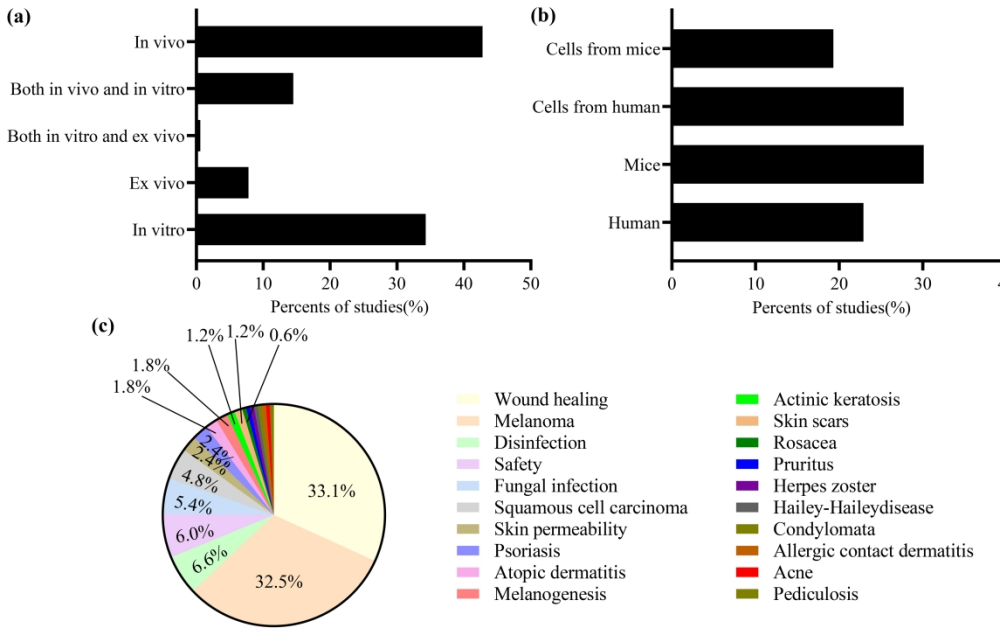
Flowchart of selection process for study inclusion.



Yearly and geographical distribution of the included studies. (a) The numbers of articles by year; (b) The world map and (c) pie chart revealed geographical distribution.



Summary of plasma sources used in dermatology. (a, b) Numbers of studies according to the type of plasma device and working gas; (c, d) Numbers of studies according to the working gas used in plasma jet and dielectric barrier discharge (DBD); (e) Percents of studies used KINPen MED, SteriPlas/MicroPlaSter and PlasmaDerm; (f) Numbers of studies according to the plasma treatment regimen (direct or indirect treatment).



Dermatological applications of cold atmospheric plasma (CAP). Percents of studies according to the methodology (in vitro, ex vivo, in vivo) (a) and the adopted treatment modalities (b). (c) Study results of indications for plasma application in dermatology.