COLD PLASMA

AN EMERGING TECHNOLOGY FOR CLINICAL USE IN WOUND HEALING

The European Wound Management Association
EWMA

Cold Plasma

An emerging technology for clinical use in wound healing

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Abbreviations

AEs	adverse events
APC	argon plasma coagulation
APDT	antimicrobial photodynamic therapy
Ar	argon
BCWG	Borderline and Classification Working Group
CAP	cold atmospheric plasma
CPT	cold plasma therapy
DBD	dielectric barrier discharges
DFU	diabetic foot ulcers
DNA	deoxyribonucleic acid
EU	European Union
FDA	US Food and Drug Administration (FDA)
FE-DBD	floating electrode dielectric barrier discharge
FGF	fibroblast growth factors
H_2O_2	hydrogen peroxide
HCPs	healthcare professionals
He	helium
HPRT	hypoxanthine phosphoribosyl transferase
MDCG	Medical Device Coordination Group
MDR	Medical Device Regulation
MMP	matrix metalloproteinases
MN	micronucleus or MN
mRNA	messenger ribonucleic acid
MRSA	methicillin-resistant *Staphylococcus aureus*
NO	nitric oxide
OECD	Organization for Economic Co-operation and Development
PAH	plasma-activated hydrogels
PAS	plasma-activated solutions
PAW	plasma-activated water
PJ	plasma jets
PLA	poly lactic acid
PMN	polymorphonuclear cells
PVA	poly vinyl alcohol
QoL	quality of life
RCTs	randomised controlled trials
RNS	reactive nitrogen species
RONS	reactive oxygen nitrogen species
ROS	reactive oxygen species
SAEs	serious adverse events
SDBD	surface dielectric barrier discharges
SOC	standard of care
TDM	transferred discharge method
TGF	tissue growth factor
UV	ultraviolet
VDBD	volume dielectric barrier discharges
VEGF	vascular endothelial growth factor
VLU	venous leg ulcers

1. Introduction and aim

Support the nature and extent of current issues facing wound management: from the policy making and healthcare system perspective.

The ongoing controversy regarding high-level evidence in wound care is well known. There is a consensus that clinical practice should be evidence-based, which can be difficult to achieve due to uncertainty about the value of the various approaches to wound management; however, we must rely on the best available evidence.

There is further fundamental confusion over the best way to evaluate the effectiveness of interventions in this complex patient population. This is, for example, illustrated by reviews of the value of various treatment strategies for non-healing wounds, which have highlighted methodological inconsistencies in primary research.[1,2]

This situation is further complicated by differences in the advice given by the regulatory and reimbursement bodies in various countries regarding both study design and how results are, and should be, interpreted. Despite this, there is an urgent need to review wound strategies and treatments to reduce the burden of care efficiently. If patients at risk of delayed wound healing are identified earlier, and aggressive interventions are taken before the wound deteriorates and complications occur, both patient morbidity and healthcare costs can be significantly reduced.

1.1 Objectives

The European Wound Management Association (EWMA) believes that cold atmospheric plasma (CAP) for wound treatment potentially represents a new, sustainable, advanced therapy, while CAP on the other hand may still have to reach its full potential. The general awareness level about CAP among healthcare professionals (HCPs) is relatively modest, which may impede the dialogue between researchers, clinicians, policy makers and payers.

This document intends to highlight and focus on technological advances in CAP for wound management, which are seen currently to be heading in several directions from a scientific, clinical and patient caregiver perspective.

When reviewing this, various critical non-clinical issues will also be discussed, especially since access to care and the evaluation of the benefits of treatment are becoming more and more a financially-driven critical factor.

The objectives of the document are to:

- review and discuss scientific evidence and clinical experiences;
- review and discuss the potentials and challenges for CAP in wound management;
- review emerging and available CAP therapies;
- discuss safety issues, reimbursement, and the regulatory framework for CAP;

About EWMA Documents

In response to the need for reviewing wound strategies and treatments, the European Wound Management Association (EWMA) has published several interdisciplinary documents[3–7] to highlight:

- the nature and extent of the problem for wound management, both from the clinical, as well as from a caregiver and patient perspective;
- evidence-based practice as an integration of clinical expertise with the best available clinical evidence from systematic research;
- the nature and extent of the problem for wound management, both from the policymaker and healthcare system perspectives.

All documents are available for free download and online reading at www.ewma.org/resource-library/

What EWMA would like to achieve

EWMA wishes to:

- increase general awareness of CAP among HCPs based on scientific knowledge;
- identify opportunities and challenges for CAP as a clinical therapy in wound healing;
- promote the development of safe and efficient solutions for the delivery of the therapy.

This aligns with the EWMA intention to be on the forefront of the development of new, sustainable, cost-effective and efficient advanced therapies, and to examine further how these measures may support the continuous improvement of wound management and promote patients' and their families' quality of life (QoL).

- supply knowledge and support for future discussions with healthcare providers and payers;
- be an inspiration for solution providers;
- call for research and actions in recommended areas if needed.

However, the document has two fundamental demarcations. It does not promote a specific intervention compared to other alternatives as this is beyond the scope for EWMA. For the same reason, non-clinical applications of CAP are not included.

1.2 Methodology

This document originates from expressions of interest by various EWMA stakeholders in an EWMA internal note which focused on the role and use of CAP in wound management. Based on a literature search conducted in PubMed and other sources, a short description of the document's aim, objectives and scope was developed during H2 2022. As a follow-up, a set of guidelines for this document, outlining General Conditions, Author Conditions and Industry Supporter Conditions was prepared in the fourth quarter of 2022. The guidelines are available upon request from the EWMA Secretariat. These two basic documents were subsequently used to identify the experts who constitute the author group.

Each author has taken responsibility for the elaboration of the first draft of a whole or part of a chapter. It has been the obligation of each author to search and investigate the relevant literature. The opinions stated in this document have been reached by a consensus of the author group, weighing their professional opinions based on their respective research, and that of their peers, as well as their own clinical experience. Several of the key opinion leaders (KOLs) and scientific high-level experts in CAP are among the authors of this document.

Therefore, a uniform search strategy was not defined, since the authors for the most part are thoroughly familiar with the existing literature. Several collaborators are also authors of reviews of various aspects of CAP for wound management.

Where there is a lack of scientific evidence, the document is based on the available literature and experts' opinions. Before its publication, the document has been reviewed by the EWMA Council, the Industrial Supporters and other stakeholders. The resulting comments have been discussed by the author group, and were either accepted or dismissed based on their scientific validity.

1.3 Structure and content

The chapters of this document were drafted and assembled to provide a logical flow, and contribute to making it accessible to HCPs and other readers, including those who are not experts in the field of CAP, for clinical use in wound management. Hence, the first chapters will present the history of CAP, review findings from the basic research on CAP, followed by chapters that describe the clinical experiences with CAP in wound management, and reflect on additional (potential) emerging CAP therapies. The last chapters will focus on the challenges an innovative wound healing therapy such as CAP can confront, and sum up the opportunities and challenges identified earlier concerning the uptake of CAP into routine clinical use for wound management.

The authors hope the document will be interesting and relevant for HCPs and other stakeholders within wound management such as solution providers, regulatory authorities, payers, procurement officers and society at large.

2. The history of CAP

2.1 What is CAP?

Physical plasma is a special gas state where atoms or molecules of a gas are excited and ionised. Plasma is often called the fourth state of matter following solid, liquid and gaseous. Plasma is generated by energy supply to a neutral gas; the application of electric fields or electromagnetic radiation is the most common method (besides chemical processes, heating or compression). Plasma generation can be realised under low pressure, atmospheric pressure and high pressure conditions. Depending on these and several other parameters, particularly the working power, plasma can be generated at low or very high temperatures. For more information and details, see Appendix I: The physics of CAP in this EWMA document.

In general, plasmas for clinical use must work at low temperatures (e.g. ±40°C at the target site during treatment) as well as in an atmospheric pressure environment. To distinguish physical plasma from the better known blood plasma in the biomedical community, several terms or amendments are in use, e.g. gas plasma, non-thermal plasma, low-temperature plasma, tissue-tolerable plasma, cold physical plasma; all of these refer to the same phenomenon. The most widespread term for plasma for medical use is cold atmospheric plasma or cold atmospheric-pressure plasma, commonly abbreviated as CAP.

Neutral gases, especially under atmospheric conditions, contain some 'background' electrons resulting from, for example, cosmic rays or radioactive radiation. By applying an electric field to a neutral gas, energy is transmitted to the electrons, which are the most mobile charged species. From this, electrons are accelerated, and they transmit energy to the neutral species by collisions. These collisions can be either elastic, resulting in no change to the internal energy of the neutral species but in a slight rise of its kinetic energy, or inelastic if electron energy is high enough. In that case, the electronic structure of the neutral species is modified, resulting in excitation or ionisation of the neutral gas atoms or molecules. Excitation means that electrons move into a higher energy state inside the atom or molecule. Most of these excited species are unstable; spontaneous de-excitation results in the emission of photons. Therefore, a plasma is visible because of the emission of light. Other excited species with longer lifetimes are called 'metastable species'; their decay by emission of radiation is hampered. In the case of ionisation, electrons are ejected from the atomic or molecular structure resulting in an electron avalanche and the generation of ions. Because of this generation of free charge carriers, plasma is electrically conductive.[8–12] A more detailed and in-depth description of the principles of CAP can be found in the Appendices of this EWMA document.

2.2 Basic types of CAP devices

For medical purposes, usually two basic types of CAP sources are tested and partially applied in medical devices – dielectric barrier discharges (DBD) and atmospheric-pressure plasma jets (PJ) (Figure 1).[13–18] A more detailed and in-depth description of the different available CAP devices can be found in Appendix II.

In DBD, plasma is ignited in a gap between a high voltage electrode and a second (counter) electrode. A dielectric (isolating) material covers one of the two or both electrodes. In the so-called volume DBD (VDBD), the target to be treated (e.g. skin wound, etc.) serves as second electrode. In this case, a direct contact between the plasma and target is realised, including a low electric current flow between the plasma device and target to be treated. Alternatively, in a so-called surface DBD (SDBD), plasma is generated around a variably designable electrode structure (e.g. circular or grid-like), which is isolated from the counter electrode which is part of the device. In this case, no direct contact of the active plasma with the target occurs and the plasma must be brought to close vicinity to the target for treatment purposes. In DBD, as working gas for plasma generation atmospheric air is usually used. Both VDBD and SDBD can be designed to generate plasmas over larger areas.[12,19]

In a so-called PJ, a two-electrode setup of variable configuration (e.g. pin electrodes, ring electrodes, plate

electrodes etc.) is mounted in or around a tube-like arrangement, in most cases inside a pen-like device. For plasma generation, a working gas flowing through the tube and the electrode arrangement is used, resulting in a so-called plasma effluent (or afterglow), which is driven out of the tube with the gas flow. In PJ, usually prefabricated gases are used, mostly noble gases – helium (He) or argon (Ar) – often doped with small amounts of molecular gases (nitrogen, oxygen, air). Because the target to be treated is not part of the primary electrode configuration, a direct plasma (effluent) contact can be realised or not, dependent on the distance between the nozzle of the tube and the target.[12,20–22] In general, PJ are preferable for localised, spot-like treatments. Large-area treatments are possible by moving the plasma effluent over the surface to be treated. To increase the treatment area, PJ arrays are possible.[23]

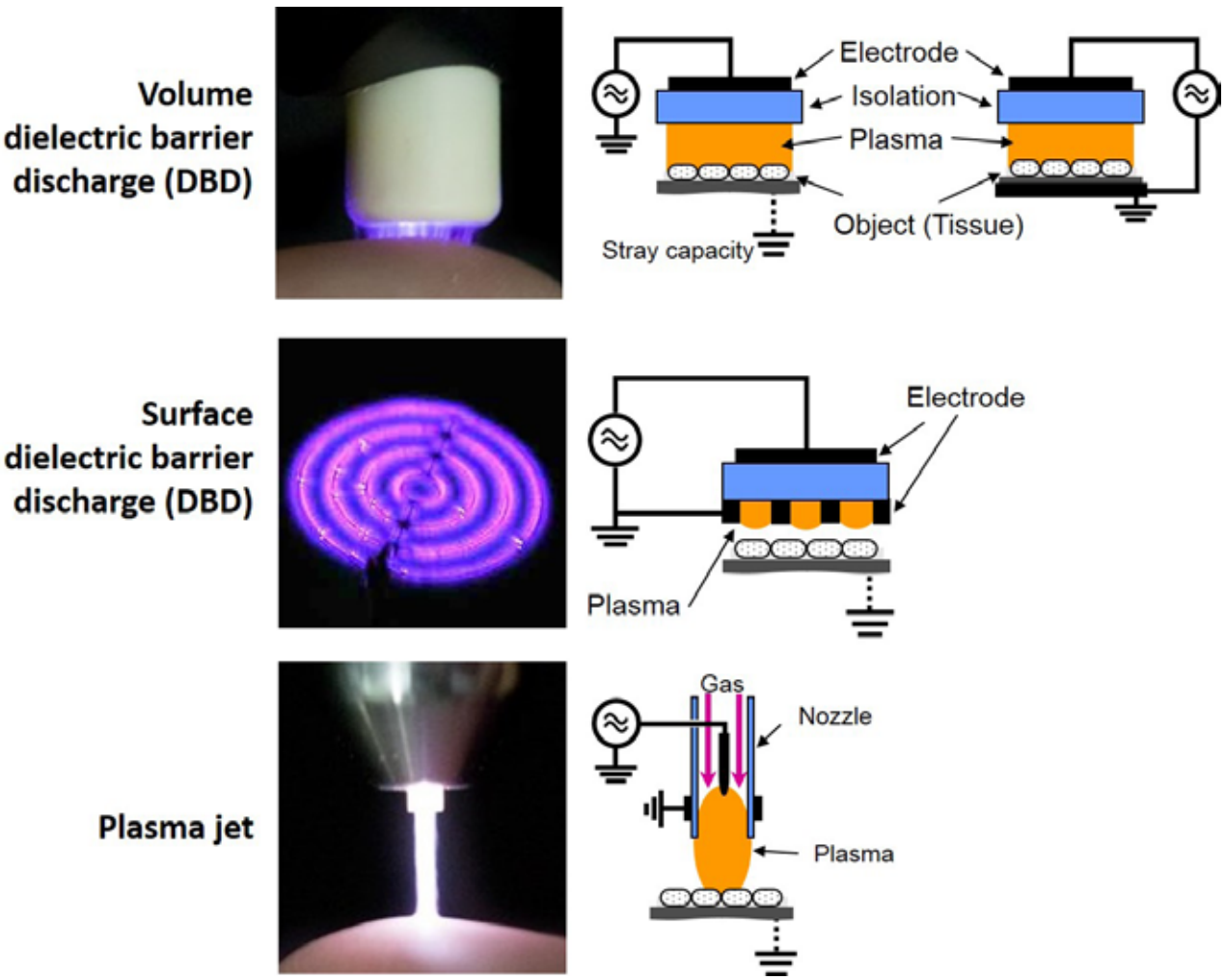

Figure 1: Basic technical principles of CAP sources for biomedical research and medical application

CAP is a multi-component system, containing ultraviolet (UV), visible and infrared radiation, electrical fields, free electrons, ions, radicals and excited gas atoms/molecules in a bulk of neutral gas atoms/molecules.[12] By interaction of electrons and high energy states of atoms or molecules with reaction partners in both the plasma phase and its close vicinity (ambient air, liquids, surfaces), secondary and tertiary reactive species are generated, which increase the complexity of the plasma 'cocktail'.[24] In the case of treatment of biological targets (cells, tissues), liquids phases play an important role in these processes. The most important and uniform feature of all CAP sources is that they work in an ambient air environment, or use ambient air as working gas. Consequently, oxygen and nitrogen-based plasma chemistry is dominating, resulting in the generation of so-called reactive oxygen species (ROS) and reactive nitrogen species (RNS), collectively known as RONS, which are considered the main components for the biological reactivity of CAP.[11,25,26] It is important to point out that the plasma state is maintained as long as the energy supply exists, i.e. it is not possible to store plasma like a conventional neutral gas. Active components are generated locally and only for the required duration of the application on-site primarily by a physical process.

2.3 General applications of CAP

Many plasma applications can be found in a wide variety of industries, mostly cleaning, etching, coating and preparation of surfaces. Here, plasma treatment optimises specific applications. These applications range from the production of integrated circuits for computers and cell phones, additive-free and low-temperature plasma-supported synthesis of chemicals and nano-materials, to the activation and hardening of material surfaces. In addition, CAP-based surface technologies are also useful for medical materials and devices such as implants, diagnostic tools or surfaces in the clinical environment, not only generating improved biocompatibility and cell attachment, but also cell-repellent and anti-bacterial characteristics. Furthermore, anti-bacterial surfaces based on CAP technologies may be also useful in clinical settings and in the immediate patient environment to prevent the transmission of infections.[27–31]

2.4 Medical applications of CAP

The same is true for another field of plasma application in the medical field – plasma-based decontamination or 'sterilisation'. A special property of CAP is that it can inactivate microorganisms and viruses without permanently influencing or destroying their surrounding structures. Consequently, this opens the way for gentle decontamination or sterilisation of sensitive and thermo-sensitive goods. Research on plasma techniques for bio-decontamination of medical devices and materials is a field of long-term research. Focused primarily on low pressure non-thermal plasma, the improved availability of CAP technology from the 1990s also led to several studies on its applicability for antibacterial treatment of medical devices, surfaces or materials.[32–36] However, sterilisation in accordance with strict regulatory criteria does not seem to be feasible with CAP, or only under special conditions.[37–39] This could be a reason why plasma-based technologies have rarely reached the level of established bio-decontamination methods. Nevertheless, there are promising application potentials, not only in medicine, hygiene and infection prevention, but also in food processing and agriculture.[40–45] The plasma applications described previously for biomaterial and implant treatment, or for decontamination purposes, can be considered such indirect medical plasma applications – materials or devices are plasma treated and subsequently supplied to a medical application.

With the direct application of physical plasma on, or in, the human body for therapeutic purposes, an innovative and interdisciplinary research field including physics, life sciences and medicine has been established within the past 15 to 20 years called 'plasma medicine'. In fact, the medical use of physical plasma in the context of so-called electro surgery, for example as argon plasma coagulation (APC), has been established for many years.[46,47] Based on the antimicrobial efficacy of CAP without influencing sensitive surfaces negatively, research was initiated to investigate CAP treatment of specific body surfaces, e.g. infected wounds or skin. First laboratory experiments on CAP application on cultivated mammalian cells demonstrated the possibility to selectively manipulate them without killing.[48,49] Together with several theoretical considerations, first experimental findings initially led to the research focus of plasma medicine on wound healing with special regard to chronic wounds.[50,51] A hypothesis

was made very early of a potential dual plasma efficacy, i.e. simultaneously deactivate wound-contaminating microorganisms and stimulate tissue regeneration directly.[52] Since its original formulation, many in vitro experiments, animal studies and clinical trials seem to confirm this hypothesis. The common consensus is that these unique properties of CAP are based most likely on effects like increased wound tissue oxygenation and vascularisation, amplified apoptosis of senescent cells, activation and/or modulation of redox signalling cascades, as well as immune cell attraction and stimulation. With continuing research, the molecular mechanisms of these effects are becoming more and more understood.[53–58]

In parallel to preclinical and clinical research on CAP in wound healing, CAP for cancer treatment has arisen as the second largest research field in plasma medicine (paragraph 5.5). First indications from in vitro studies of a possible increased sensitivity of cancer cells to CAP compared to non-malignant cells – a kind of 'selectivity' – led to high expectations of bringing about a "paradigm shift in cancer therapy".[59] Meanwhile, many in vitro experiments as well as experimental animal studies with a great number of cancer cell lines were realised to evaluate the potential of CAP in oncological treatment.[60–62] Basic molecular mechanisms of CAP on cancer cells are considered to comprise of both a direct cancer cell killing by apoptosis, and indirect (systemic) effects by initiation of immunogenic cell death (paragraph 5.5).[63,64] Despite experimental data, clinical applications are rare, caused by open questions with regard to the safety of CAP application for cancer treatment. In particular, it should be clarified whether stimulating plasma effects, which form the basis of tissue regeneration in wound healing, can lead to metastases in the case of sub-effective treatment of cancer tissue.[65] Furthermore, due to the limited penetration depth of CAP, any treatment of bulk tumours is limited by its efficacy (paragraph 5.7).

3. Review of basic research on CAP

3.1 Cold plasma and wound healing in vitro

Cold plasma provides a rich source of ROS and RNS, collectively known as RONS. These are, as the name suggests, highly reactive molecules that can readily oxidise biomolecules. Historically, RONS were thought to be harmful and associated with free radical ageing and disease. Currently, it is widely recognised that RONS are essential for the maintenance of good health through their participation in a plethora of cellular signalling pathways that aid wound healing and help the body fight infections.[66] Normally, this occurs through a process called redox homeostasis, where cells balance the generation and consumption of RONS to maintain function of redox-sensitive signalling proteins.[67]

Redox homeostasis is disturbed usually in slow healing wounds; this contributes to cell death and poor cell function that ultimately delays, or even prevents, healing.[68] Since CAP is a rich source of RONS, it can be theoretically used to restore the redox balance in these wounds to promote cellular activity and healing.[69] In addition to healing, CAP also has a strong broad-spectrum antibacterial property. This makes CAP suitable for targeting all stages of wound healing, from infection control through to tissue regeneration. The following subsections provide a summary of the in vitro experiments to investigate the antibacterial activity of CAP in wound decontamination in order to elucidate the underlying mechanisms of how CAP regulates cellular activity in wound healing, and how to tailor CAP devices specifically for wound treatment.

3.1.1 Broad-spectrum antibacterial activity of CAP

CAP has been shown to be effective against a broad-spectrum of wound pathogens, including *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*,[70–77] and antimicrobial resistant strains such as methicillin-resistant *S. aureus* (MRSA),[78–80] demonstrating a strong antibacterial efficacy of CAP in wound care. Apart from treatment of planktonic and single colony bacteria, CAP has been shown to be effective at reducing growth of bacterial biofilms in vitro.[71,81–87] The reason why CAP can target a broad-spectrum of bacteria and mature biofilm infections is attributed to its unique chemical and physical mode-of-action. CAP readily produces nitric oxide (NO), which is known to disrupt quorum sensing.[88] This can help break down the biofilm architecture, enabling penetration of other antibacterial agents produced by CAP, such as hydrogen peroxide (H_2O_2), which is a widely recognised effective disinfectant.[89] In addition to its chemical action, the physical components of CAP, such as UV irradiation, further enhance its antimicrobial efficacy.[70]

3.1.2 Major components of CAP implicated in wound healing

CAP readily generates a heterogeneous mixture of RONS, which are considered beneficial for wound healing. Amongst these RONS, i.e. H_2O_2 and NO, have arguably been most widely studied due to their well-known role in the regulation of cellular signalling processes, which stimulate wound healing.[90–96] Furthermore, H_2O_2 and NO have also been linked to enhancing cell proliferation in vitro.[97–100] In addition to these, CAP produces a variety of other molecules which can also stimulate different processes in wound healing.

3.1.3 Cellular mechanism of CAP in wound healing

CAP has been shown to upregulate growth factor production in cells, including vascular endothelial growth factor (VEGF),[101] fibroblast growth factors FGF-2,[102] FGF-7,[103] and alter integrin expression[104] to stimulate cell proliferation and migration. Given the heterogeneous mixture of RONS generated by CAP, it is likely that CAP can be used to target many cellular pathways to stimulate growth. Although some insights into the biomolecular mechanisms of CAP in wound healing have been gained through the use of transcriptomics[105] and proteomics,[106] this research is still in its infancy, and much more research is still required to elucidate the major cellular pathways regulated by CAP in wound healing.

3.1.4 Oxygenation of CAP in wound healing

Oxygen is a key requirement to achieving a good wound healing outcome.[107,108] Therefore, methods to enhance blood flow and oxygenation at the wound site can help to stimulate healing, particularly in ischaemic wounds

characterised by poor blood flow. CAP has been shown to promote angiogenesis[109] and oxygenate hypoxic solutions.[110] CAP can oxygenate tissues through the conversion of some of the RONS (e.g. H_2O_2) into oxygen, while it also generates NO, an important molecule associated with CAP-induced vasodilation, blood flow, and increased tissue oxygenation.[111,112] In vitro experiments using simple models of biological tissues constructed from gelatine, agarose and simple mimics of biological fluid such as water, phosphate buffered saline and cell culture media, have shown that CAP treatment directly influences the concentration of dissolved oxygen.[110,113–115] However, these results also showed that CAP has the potential to oxygenate or deoxygenate, depending on the start oxygen concentration in the biological target. An explanation for this is that CAP produces molecular oxidants which convert into molecular oxygen side products when the oxidants decay in solution.

Configurations such as PJ are operated typically with an inert gas such as He or Ar. Since only a very small fraction of the gas is ionised (typically 10–4–10–7%),[116] the neutral He and Ar gas can readily displace molecular oxygen in a process referred to as sparging.[110,117] These in vitro findings could be important for the clinical treatment of wounds, since they suggest that CAP treatment has the potential to both stimulate healing through oxygenation, but also conversely impair healing through deoxygenation, depending on the starting oxygen concentration in the wound.

3.1.5 Tailoring CAP for wound treatment

With wound healing processes, the concentrations of H_2O_2 and NO are elevated generally at the early inflammatory stages to recruit neutrophils and macrophages to fight infection. Afterwards, these molecules reduce in concentration to allow infiltration of lymphocytes, fibroblasts and keratinocytes to close the wound.[25] Chronic wounds with delayed healing, however, typically have an imbalance of RONS that impairs the ability of the body to clear infection or heal the wound. Therefore, restoring the optimal RONS and oxygen balance can potentially be useful to help the body fight infections, and stimulate healing for these wounds.

The dose and chemical composition of RONS produced by CAP can be tailored to change its bacteriocidic or cell stimulating effect. For example, the ratio of ROS:RNS can be tuned through variation of the gas composition interacting with the plasma discharge to optimise CAP treatment for bacterial inactivation[118] or cell growth.[119] CAP can be humidified, thus moistening wounds while also promoting the production of certain ROS, such as H_2O_2, to regulate cell growth.[120] In addition, the composition of RONS produced by CAP can be controlled through electrical manipulation of the plasma discharge; this is most easily done with CAP sources operated with bipolar pulsed direct current (DC) power supplies. Such power supplies can be used to control the production of ROS:RNS by switching the polarity of the applied voltage.[121] Furthermore, RONS and oxygen concentrations can also be regulated in vitro by cycling the CAP treatments under different modes of operation.[122]

Whilst the potential to influence RONS concentration and composition with CAP sources has been demonstrated, there is still a lot more work to be done to elucidate how the physicochemical properties of CAP are influenced by operational parameters and the surrounding environment. This work needs to be completed to provide knowledge on how the RONS' composition can be tuned to wounds specifically. More details can be found in Appendices I and II of this EWMA document.

3.2 Cold plasma and wound healing in vivo

The healing potential of CAP has been investigated in vivo in numerous studies using various animal models. The majority of CAP preclinical studies have been conducted in small size rodent models (mice and rats),[123,124] although other medium size and large animals, such as rabbits, pigs and sheep, have also been used.[56,125] To test the efficacy of CAP in wound healing, various types of PJ devices (e.g. various commercial devices, custom lab-based CAP jets, Ar CAP jets, He CAP jets, air CAP devices and jets using inert gas mixed with O_2) and treatment regimes (e.g. direct plasma treatment, using plasma-treated solutions indirectly, plasma plume in contact with the tissue, plasma plume not in contact with the tissue) have been explored (Table 1).

Some studies also assess the effect of CAP treatment in combination with standard care (e.g. hydrocolloid dressings) and other experimental therapies including honey and zinc oxide (Table 1). In terms of wound types, most studies focussed on the effect of CAP in acute (non-diabetic) wounds. There are studies, however, which extend to infected wounds, wounds in diabetic mice,

Table 1: Animal studies on CAP wound healing.

Study*	Animal	Wound	Plasma	Key outcomes
Nastuta et al., 2011[126]	Wistar rat	5mm burn wound w/ sulphuric acid (dorsum)	He PJ	Increased white blood cell count in first 3 days. Histology showed abnormal epidermis and subjacent dermis regeneration. Local oxidative stress increased. Epidermis re-epithelialisation accelerated; superficial dermis recovery slowed.
Ermolaeva et al., 2011[71]	Adult male Sprague Dawley rats	Removal piece of skin and subcutaneous oedema. Fresh wound covered with cotton pellets impregnated with mixed culture of *P. aeruginosa* and *S. aureus* for 3 days.	Ar plasma torch	Results demonstrated considerable potential of non-thermal Ar plasma in eliminating pathogenic bacteria both in vitro and in vivo. Results obtained suggested that, in general, non-thermal Ar plasma is more effective against Gram-negative than Gram-positive bacteria.
Yu et al., 2011[127]	Balb/c mouse	6mm punch (dorsum), sterile and infected groups (*P. aeruginosa*)	$He/O_2/N_2$ PJ	Improved re-epithelialisation and neovascularisation. Decreased microbial burden.
Arndt et al., 2013[128]	129Sv/Ev mouse	6mm punch (dorsum)	Ar plasma torch	Accelerated wound closure. Increased neutrophil and macrophage count, increase in pro-inflammatory factors.
Garcia-Alcantara et al., 2013[129]	Balb/c mouse	6–0mm long and 4–5mm deep incision (leg)	He and Ar PJ	Sequential Ar and He treatment accelerated coagulation and healing significantly more than untreated wound.
Wu et al., 2013[130]	Yorkshire pig	$20mm^2$ abrasion (1mm deep)	Air DBD	Intact skin damaged at higher power and longer exposure times; lower power/time cause no harm. Additional coagulation seen in plasma treated wounded tissue. Wounded skin exposed to high power plasma developed burns.
Jacofsky et al., 2014[131]	BKS. Cg–+Leprdb +Leprdb OlaHsd mouse	15x10mm skin removal w/ surgical scissors (dorsum)	He PJ	Accelerated wound closure; 60 second treatment heals faster than 30- and 90-second groups. Healing rates equalised by 15 days. Plasma treatment is most effective during early stages.
Ngo Thi et al., 2014[132]	C57BL/6JNarl mouse	5mm diameter 2nd degree burn w/ Al bar (dorsum)	N_2/Ar PJ	Accelerated wound closure. Enhancement of angiogenesis via increase in blood flow and CD31.
Nasruddin et al., 2014[133]	Balb/c mouse	2mm punch (dorsum)	Ar PJ	Accelerated wound closure days 3–8. Greater re-epithelialisation rate days 3–7. At later stages, neutrophil and macrophage count lower than control.
Nasruddin et al., 2015[134]	Balb/c mouse	4mm punch (dorsum)	Ar PJ	Plasma + water improves wound contraction during inflammation and granulation phases of healing.

** Articles sorted by year of appearance.*

Study*	Animal	Wound	Plasma	Key outcomes
Ngo et al., 2015[135]	C57BL/6JNarl mouse	Burn wound w/ Al bar (dorsum)	N_2/Ar PJ	Accelerated wound closure.
Kim et al., 2015[136]	SKH-1 mouse	5mm punch (dorsum)	Ar/Air PJ	Accelerated wound closure, particularly in early stages. Ar/Air more effective than Ar. Increased thickness of epidermis. No damage induced by PJ. Ar/Air plasma enhances IL-6 and TGF-β1.
Xu et al., 2015[137]	Balb/c mouse	4mm punch (dorsum)	Ar PJ	50-second treatment had negative effect on wound healing compared to control. Shorter treatment times accelerate wound closure, promote regeneration and maturation of epidermal cells and blood vessels.
Shao et al., 2016[138]	C57BL/6JNarl mouse	3x20mm CO_2 laser (dorsum)	N_2/Ar PJ	Promotion of wound healing in wound bed and accelerated wound closure. Enhancement of blood flow.
Hung et al., 2016[139]	SD rat	2x2mm skin removal (dorsum)	Ar/O_2 DBD	Accelerated wound closure. Shorter healing time. No evidence of organ toxicity
Kim et al., 2016[140]	Balb/c mouse	1.5mm punch, ear	Ar PJ	Increased angiogenesis in early stages of wound healing. Vascular wound area of plasma treated wound significantly decreased compared to control.
Schmidt et al., 2017[141]	SKH1-hr mouse	3mm^2 skin removal w/ micro scissors (ear)	Ar PJ	Accelerated wound re-epithelialisation days 3–9.
Nasruddin et al., 2017[142]	Balb/c mouse	4mm punch (dorsum)	Ar PJ	Wound sizes significantly lower than in control days 4–13. Earlier wound healing for plasma treatments than control. No additional affect from combination of plasma/honey in dressing.
Kos et al., 2017[143]	Balb/c mouse	Intact skin	He PJ	Skin damage (burns) was noticed immediately after jet treatment. Further damage 24–38 hour after treatment. Longer treatment times resulted in more damage. Higher flow rates resulted in more skin damage. Jet temperature noted to reach >90°C
Schmidt et al., 2017[144]	SKH-1 mouse	3mm^2 skin removal w/ micro scissors (ear)	Ar PJ	No long-term systemic signs of pro-inflammatory cytokines, tumour marker levels. No long-term signs of abnormality in organs.
Shahbazi Rad et al., 2018[145]	Balb/c mouse	6mm punch (dorsum)	He PJ	Accelerated wound closure. Treatments at 30/40 seconds healed faster than other groups.
Arndt et al., 2018[146]	Sv/Ev mouse	6mm punch (dorsum)	Ar plasma torch	Promotes angiogenesis during wound healing. CD31 and FGF-2 mRNA expression enhanced.
Choi et al., 2018[147]	HRM2 mouse	Intact skin	Ar DBD	No signs of tissue damage. Significant increase in epidermal thickness and dermal collagen density. Increased growth factor expression.
Duchesne et al., 2018[148]	Balb/c mouse	6mm punch (dorsum)	He PJ	10s direct treatment (single or three times) and single 30 seconds showed no positive or negative effect on wound closure. Direct treatment of 30 seconds every 2 days decreased wound closure rate.

Study*	Animal	Wound	Plasma	Key outcomes
Wahyuningtyas et al., 2018[149]	Balb/c mouse	4mm punch (dorsum)	Ar PJ	Direct plasma shows signs of dryness and necrosis of wound; not seen in control or plasma and dressing containing honey dressing. Smaller wound areas were observed with plasma and honey combination compared to control.
Duchesne et al., 2019[150]	Balb/c mouse	10mm 3rd degree burn with brass block (dorsum), skin graft applied	He PJ	Increased angiogenesis. Accelerated wound healing.
Darmawati et al., 2019[151]	Balb/c mouse	4mm punch (dorsum)	Gas PJ	Plume in contact caused damage to wound tissue and impeded healing. Non-contact promoted wound healing and re-epithelialisation.
Shahbazi Rad & Davani, 2020[152]	Balb/c mouse	6mm punch (dorsum)	SDBD / Ar PJ / He PJ	Accelerated wound closure. He PJ treatment resulted in fastest wound healing compared to Ar jet or DBD.
He et al., 2020[153]	db/db C57BL mouse	6mm punch (dorsum)	He PJ	Accelerated wound closure in treatment groups. Decrease in expression of IL-6, tumour necrosis factor-α, inducible NO synthase; increase in VEGF and growth factor-β and superoxide dismutase.
Xu et al., 2020[154]	ICR mouse	2cm diameter skin removal (dorsum), inoculated with *P. aeruginosa*	PAW (DBD device)	Accelerated wound closure and shorter time to heal. Lower counts of inflammatory cells. No significant difference in organ function.
Amini et al., 2020[155]	Wistar rat	Diabetes induction, 10mm punch (dorsum), inoculated w/ *S. aureus*	He PJ	Accelerated wound healing. Increased epithelium formation and lower inflammatory cell count than control.
Martines et al., 2020[156]	Bergamasca sheep	4x4cm scalpel full thickness wound	He plasma torch (indirect) (array of 16 sources)	Accelerated wound closure. Reduction in bacterial load. Inflammatory stage reduced compared to control. Increased VEGF expression anticipating blood vessel formation.
Wang et al., 2021[157]	ICR mouse	2cm diameter skin removal (dorsum), inoculated with *P. aeruginosa*	PAW (DBD device)	PAW treatment showed accelerated wound closure compared to control and alcohol treatment. Pro inflammatory cytokine expression higher at early stages, lower at later stages. VEGF expression higher. No significant difference in blood biochemical index or organ tissue structure.
Dang et al., 2021[158]	C57B/6 mouse	1cm diameter 2nd degree burn w/ Al bar (dorsum). Sterile and infected groups (*S. aureus*)	Ar PJ	Accelerated healing in sterile and infected burn wounds. Lower TNF-α levels. Bacterial burden unchanged.
Schmidt et al., 2021[159]	SKH1 mouse	3mm^2 skin removal w/ micro scissors (ear)	Ar PJ	Increase of oxygen saturation and perfusion. Increased haemoglobin concentration. Decreased water content.

Study*	Animal	Wound	Plasma	Key outcomes
Schmidt et al., 2021[55]	SKH1-hr mouse	3mm^2 skin removal w/ micro scissors (ear)	Ar PJ	Enhanced oxygenation and haemoglobin perfusion of superficial skin layer.
Darmawati et al., 2021[160]	Balb/c mouse	4mm punch (dorsum), inoculated with *S. aureus*	Gas PJ	Plasma contact with the wound reduced infection, but inhibits wound healing. Non-contact plasma treatment mode is not effective in eliminating biofilms and impeded healing. Contact mode days 0–7 and non-contact mode days 8–14 able to remove biofilm and stimulated wound healing.
Evert et al., 2021[161]	B6C3F1 mouse	Intact mucosa	2 sources: Ar PJ and He/N_2 PJ	1 year of repeatedly applied CAP treatment to oral mucosa was well tolerated. Plasma treatment showed no increase in mortality or carcinogenicity.
Amini et al., 2021[162]	New Zealand white rabbit	Diabetes induction. 2mm incision and tendon cut	Gas PJ	Plasma at 5kV showed no effect. Plasma at 10kV shows accelerated healing. Lower presence of inflammatory cells with plasma compared to control.
Melotti et al., 2021[163]	Bergamasca sheep	4x4cm scalpel full thickness wound	He plasma torch (indirect) (array of 16 sources)	Combinatory therapy of mesenchymal stem cells and plasma leads to slower but more effective wound healing.

burns, and wounds from x-ray irradiation. In mice studies, the typical sample size ranged from 5–40 mice, assigned mainly into two groups – an experimental group wherein the wound was directly exposed to CAP, CAP-treated liquid, or CAP combined with other therapies; and a control group wherein the wound was left untreated or treated with a therapy without CAP. In some studies, each mouse served as its own control (i.e. each of two wounds at different locations in a mouse served as a control and experimental arm) while, in others, separate mice were used for control and experimental group.

The majority of studies on the effects of CAP in wounded mice point to: accelerated wound closure;[128,129,131,134–136,138,139,141,145,152–154] improved angiogenesis, re-epithelialisation and vascularisation;[132,140,142,146] neutrophil and macrophages infiltration;[128,132,133] elevated levels of cytokines and growth factors;[146,147,155,157,158] oxygenation of tissue;[55,159] and reduced microbial burden.[126,127,157,157,160] However, it is worth highlighting that, in some studies, a higher rate of wound closure and accelerated re-epithelialisation were observed only in the early phase of wound healing, while in later stages, at the end of the observation period, wounds closed completely similarly in both CAP-treated wounds and untreated wounds.[128,133,140,141] A few studies showed that the CAP exposure had no effect on wound healing (Table 1).[140,142,148,149]

In summary, the healing effects of CAP treatment were more evident using moderate or shorter treatment times,[131,137] pointing towards a dose dependence and the possibility to 'over treat' a wound. For example, prolonged treatment and/or treatment when the CAP plume is in contact with the skin/wound has been shown to have deleterious effects such as dehydration, hypoxia and skin damage.[137,143,149,150] When the CAP plume is in contact with the wound, Darmawati et al. showed an increase in bacterial killing,[160] but it is cautionary to note that the plasma plume contact showed significant damage to wound tissue.[151] However, both studies demonstrated a faster healing of the wound with non-contact regimes. Investigators observed that optimising each CAP device, and determining an optimum threshold dose of CAP exposure, overcomes undesirable effects of CAP, while still inducing beneficial effects such as disinfection and wound healing.

Other solutions include using CAP-activated liquids[154] or introducing hydrogels/dressings along with CAP

treatment.[149] Various studies to date report considerable variation in results, which most likely is caused by a lack of standardisation in method. Current literature refers to a range of different types of CAP devices and different treatment modalities (i.e. direct, indirect, contact, non-contact, different gases). Nevertheless, so far, no significant adverse side effects of CAP treatments in vivo have been observed (Table 2).

A study by He et al. showed that CAP treatment did not lead to any damage to the mice's skin, nor to liver and kidney function, days after the CAP treatment.[153] Similarly, Evert et al. reported no carcinogenic effects in mice after repeat treatments 3 times per week over a 12 month period,[161] while Schmidt et al. observed no inflammatory or carcinogenic effects 350 days after CAP wound treatment,[144] highlighting the long-term safety of the used CAP devices.

Besides mice, CAP has also shown positive effects on wound healing in larger animal models. For example, CAP improved neovascularisation and collagen production in

Table 2: Plasma devices used for in vivo studies of wound healing.

Study*	Plasma	Device	Mode of operation
Nastuta et al., 2011[126]	He PJ	In-house developed device	40 seconds daily
Yu et al., 2011[127]	He/O_2/N_2 PJ	In-house developed device	10 minutes daily
Arndt et al., 2013[128]	Ar plasma torch	MicroPlaSter ß (Adtec Healthcare Ltd)	2 minutes daily
Garcia-Alcantara et al., 2013[129]	He and Ar PJ	In-house developed device	1 minute / 5 minutes; repeat treatments of Ar followed by incision (three repeats of 1 minute), followed by He treatment (three repeats of 5 minutes)
Wu et al., 2013[130]	Air DBD	In-house developed device	30 seconds – 15 minutes; single treatment
Jacofsky et al., 2014[131]	He PJ	In-house developed device	30/60/90 seconds; 1x or 2x daily
Ngo Thi et al., 2014[132]	N_2/Ar PJ	In-house developed device	Daily
Nasruddin et al., 2014[133]	Ar PJ	In-house developed device	1 minute daily
Nasruddin et al., 2015[134]	Ar PJ	In-house developed device	1 minute daily
Ngo et al., 2015[135]	N_2/Ar PJ	In-house developed device	Daily
Kim et al., 2015[136]	Ar/Air PJ	In-house developed device	5 minutes daily
Xu et al., 2015[137]	Ar PJ	In-house developed device	10/20/30/40/50 seconds daily
Shao et al., 2016[138]	N_2/Ar PJ	In-house developed device	1 minute; single treatment or on days 1,2,3
Hung et al., 2016[139]	Ar/O_2 DBD	Taiwan Yih Dar Technologies	5 minutes daily
Kim et al., 2016[140]	Ar PJ	In-house developed device	2 minutes daily
Schmidt et al., 2017[141]	Ar PJ	kINPen 11 (neoplas med GmbH)	20 seconds daily
Nasruddin et al., 2017[142]	Ar PJ	In-house developed device	2 minutes daily
Kos et al., 2017[143]	He PJ	In-house developed device	30/60/120/180/240 seconds; single treatment
Schmidt et al., 2017[144]	Ar PJ	kINPen 11 (neoplas med GmbH)	20 seconds daily
Shahbazi Rad et al., 2018[145]	He PJ	In-house developed device	10/20/30/40/50 seconds daily

** Articles sorted by year of appearance.*

Study*	Plasma	Device	Mode of operation
Arndt et al., 2018[146]	Ar plasma torch	MicroPlaSter ß (Adtec Healthcare Ltd)	2 minutes daily
Choi et al., 2018[147]	Ar DBD	In-house developed device	5 minutes; 6 treatments over 2 weeks
Duchesne et al., 2018[148]	He PJ	In-house developed device	10 or 30 seconds. Every 24 or 48 hours
Wahyuningtyas et al., 2018[149]	Ar PJ	In-house developed device	1 minute daily
Duchesne et al., 2019[150]	He PJ	In-house developed device	30 seconds every 48 hours
Darmawati et al., 2019[151]	Gas PJ	In-house developed device	3 minutes daily
Shahbazi Rad & Davani, 2020[152]	SDBD / Ar PJ / He PJ	In-house developed device	30 seconds daily
He et al., 2020[153]	He PJ	In-house developed device	90/180 seconds daily
Xu et al., 2020[154]	PAW (DBD device)	In-house developed device	5 minutes (PAW); applied on days 0,4,7,10
Amini et al., 2020[155]	He PJ	PLASMA TEB, model Saion 88	3 minutes daily
Martines et al., 2020[156]	He plasma torch (indirect) (array of 16 sources)	In-house developed device	2 minutes daily
Wang et al., 2021[157]	PAW (DBD device)	In-house developed device	5 minutes (PAW); PAW applied on days 0,1,2,4,8
Dang et al., 2021[158]	Ar PJ	In-house developed device	30 seconds daily
Schmidt et al., 2021[159]	Ar PJ	kINPen MED (neoplas med GmbH)	10 seconds every third day
Schmidt et al., 2021[55]	Ar PJ	kINPen MED (neoplas med GmbH)	3 seconds, 3 times weekly
Darmawati et al., 2021[160]	Gas PJ	In-house developed device	3 minutes daily
Evert et al., 2021[161]	Ar PJ and He/ N2 PJ	kINPen 09 (neoplas med GmbH) & In-house developed device	10/60 seconds (kINPen) 8/48 seconds (He/N2 jet); 1/month for 1 year
Amini et al., 2021[162]	Gas PJ	In-house developed device	30 seconds
Melotti et al., 2021[163]	He plasma torch (indirect) (array of 16 sources)	In-house developed device	2 minutes daily

rabbits[162], wound healing and an increase in proliferation and growth factors in sheep,[156,163] and blood coagulation in wounded pigs.[130] Similar to the results in mice, extended CAP exposure durations can damage the intact and wounded skin in pigs, while shorter treatment times did not.[130]

To conclude, there exists ample preclinical evidence to show the potential of CAP devices in wound healing; however, studies indicate that optimisation of the operation and treatment parameters is essential for each plasma device. Whilst most studies to date have been conducted in small murine models, a number of studies were done with larger animals. These models remain relevant as studies progress towards human trials, helping to unravel mechanistic understanding of CAP interventions.

4. The clinical perspectives of CAP in wound healing

4.1 Clinical evidence for the efficacy of CAP to promote healing of chronic wounds

As discussed in previous chapters, CAP is a partially ionised gas, and consists of multiple components including, among others, RONS, electromagnetic fields, as well as UV, visible and infrared radiation.[12] This cocktail of different components acts synergistically and has two major properties that are considered to promote tissue regeneration and wound healing. On the one hand, CAP inactivates microorganisms efficiently, including multi-resistant bacteria. On the other hand, it stimulates migration, as well as proliferation of eukaryotic cells, and increases micro-circulation in treated tissues.[164–170]

The treatment of chronic wounds is only one of many different potential medical applications of CAP.[171] However, it is the most studied field, with the most elaborate clinical evidence for the efficacy and safety of CAP. Early pilot studies that led to the certification of the first plasma devices have, in the meantime, been complemented with a series of structured case reports and several randomised controlled trials (RCTs) (summarised in Table 3).

The initial investigations focused primarily on demonstrating the safety of various CAP devices and their ability to decrease bacterial load in persistent wounds.[166,170,172,175] For example, two prospective RCTs evaluating the application

Table 3: Overview of clinical studies assessing the efficacy of CAP in the treatment of chronic wounds.

Study	Study type	Plasma tech-nology	Subjects	Wound type	Aim of study/ endpoint(s)	Main conclusions
Isbary et al. 2010[166]	Prospective clinical RCT	Plasma Torch (Ar)	36	Chronic infected wounds	Safety and efficiency to decrease bacterial load	Highly significant reduction in bacterial load.
Isbary et al. 2012[172]	Prospective clinical phase II RCT	Plasma Torch (Ar)	24	Chronic infected wounds	Safety and reduction in bacterial load	MicroPlaSter α: Significant reduction in bacterial load. MicroPlaSter β: Highly significant reduction in bacterial load.
Isbary et al. 2013[173]	Open retrospective clinical RCT	Plasma Torch (Ar)	70	Chronic infected wounds	Effects on wound healing (secondary endpoint)	Wound healing may be accelerated by CAP, particularly for chronic venous ulcers.
Daeschlein et al. 2015[165]	One-armed interventional clinical study	PJ (Ar)	11	Chronic leg ulcers	Antimicrobial efficacy	Ar-based CAP serves as a potent treatment modality to limit multi-drug resistant microbial colonisation.
Klebes et al. 2015[170]	Three-armed interventional clinical study	PJ (Ar)	34	Chronic leg ulcers	Reduction in bacterial load	Combined use of CAP and conventional antiseptics might represent the most efficient strategy for antiseptic treatment of chronic wounds.

Study	Study type	Plasma technology	Subjects	Wound type	Aim of study/ endpoint(s)	Main conclusions
Brehmer et al. 2015[174]	Monocentric, two-armed, open, prospective, clinical RCT	VDBD (air)	14	Chronic VLU	Safety (primary) and efficacy and applicability (secondary)	CAP treatment with the DBD device is safe and effective in patients with chronic VLU.
Ulrich et al. 2015[175]	Monocentric, two-armed interventional pilot study	PJ (Ar)	16	Chronic leg ulcers	Antimicrobial effects	Immediate antimicrobial effects of CAP plasma almost comparable to octenidine without signs of cytotoxicity.
Chuangsuwanich et al. 2016[176]	Prospective, clinical RCT	PJ (Ar)	50	Pressure ulcers	Primary: wound healing effect (size, amount of exudate, wound base); secondary: reduction in bacterial load	CAP treated group had significantly better PUSH (Pressure Ulcer Scale for Healing) scores and exudate amount.
González-Mendoza et al. 2020[177]	Non-controlled clinical trial	DBD (He)	32	Chronic venous and mixed ulcers	Efficacy of non-thermal He plasma (wound size)	The non-thermal plasma generated in a needle reactor with He gas is a promising candidate for therapeutic use in the treatment of neuropathic leg ulcers. Application to patients with autoimmune diseases was not beneficial.
Stratmann et al. 2020[178]	Prospective, randomised, placebo-controlled, patient-blinded clinical trial	PJ (Ar)	45	DFU	Primary: reduction in wound size, clinical infection and microbial load; secondary: time to relevant wound reduction (>10%), reduction of infection, parameters of patients' well-being, and treatment-associated AEs	CAP results in wound surface reduction and reduced time to wound closure independent from background infection.
Mirpour et al. 2020[179]	Randomised, parallel, 2-group clinical trial	PJ (He)	44	DFU	Wound size, number of cases reaching wound size of <0.5, and bacterial load	CAP accelerates wound healing in DFU, with immediate antiseptic effects that do not seem to last long.
Amini et al. 2020[180]	Clinical RCT	PJ (He)	44	DFU	Effect on inflammatory factors	CAP has beneficial effects on the inflammatory phase of DFU.

Study	Study type	Plasma technology	Subjects	Wound type	Aim of study/ endpoint(s)	Main conclusions
Moelleken et al. 2020[181]	Three-armed randomised controlled clinical pilot study	Plasma Torch (Ar)	37	Therapy-refractory chronic wounds	Can comparable good results be achieved with CAP treatment 1x/week compared to 3x/ week	CAP improves various aspects of wound healing in patients with therapy-refractory chronic wounds. A more frequent treatment (3×/week) had no advantage over the less frequent (1×/week) treatment.
Jensen et al. 2021[167]	Prospective, controlled clinical cohort trial	VDBD (air)	20	Chronic leg ulcers due to diabetes mellitus or peripheral artery disease	Effects of a repetitive CAP application on micro-circulation	Repetitive application of CAP boosts and prolongs tissue oxygen saturation and capillary blood flow in chronic wounds compared to a single application.
Samsavar et al. 2021[182]	Investigator-blind, clinical RCT	PJ (He)	20	DFU	Efficacy and safety	CAP is an effective treatment option for DFU in terms of wound surface reduction and antibacterial effects.
Schleusser et al. 2022[183]	Prospective, controlled clinical cohort trial	VDBD (air)	20	Chronic wounds on the lower extremity	Effect on micro-circulation	CAP increases micro-circulation parameters in chronic wounds significantly.
Strohal et al. 2022[184]	Multicentre randomised controlled, open-label, non-inferiority clinical trial	PJ (Ar)	78	Infected or non-infected chronic wounds of different aetiology	Sum of granulation tissue, wound area reduction, healing rate, time to complete healing, changes in wound pH value, infection score, exudate level and local tolerability	Treatment with CAP-jet appeared not only non-inferior, but even superior to BP in all wound entities analysed with a favourable tolerability profile. CAP significantly accelerates wound closure. Patients in the CAP treatment group required significantly less antibiotic therapy and experienced a significant reduction in wound pain and improved QoL.
Wiegand et al. 2022[185]	Multicentre prospective, randomised clinical trial	PJ (Ar)	15	DFU	Primary: reduction of wound size over trial period; secondary: decrease of bioburden, inflammatory processes, subjective pain relief	Over course of treatment, wound size reduced by 45.5% vs. SOC 25.0% (p=0.0618). Six patients exhibited reduction in bacteria, four treated, two SOC / control. In other cases, no change was detected from day 1 to day 29. Two SOC patients showed increased Gram staining. Several patients experienced wound pain decrease.
Lagrand et al. 2023[186]	Non-controlled clinical trial	VDBD (air)	20	DFU	Safety (primary), bacterial load and wound size (secondary)	CAP treatment in DFU was safe and well tolerated. Ulcer size and *S. aureus* colonisation decreased during treatment.

Study	Study type	Plasma tech-nology	Subjects	Wound type	Aim of study/ endpoint(s)	Main conclusions
Abu Rached et al. 2023[187]	Multicentre clinical RCT	SDBD (air)	48	Chronic, non-healing arterial or venous wounds on the lower leg	Primary: relative reduction of the wound area after the 4-week treatment period; secondary: events of complete wound closure, changes in wound tissue quality, bacterial load, occurrence of wound relapse, wound pain, QoL, and necessity of hospitalisation	CAP therapy significantly accelerates wound closure compared with standard wound treatment. Complete wound closure was exclusively observed in the CAP group during the intervention period.
Bakker et al. 2024[188]	Three-armed open label RCT	VDBD (air)	46	Chronic VLU	Percentage wounds healed (primary), wound area reduction, SAEs	Direct-CAP applied once or twice a week improves VLU wound healing in primary care.
Ligresti et al. 2024[189]	Single-arm open-label observational multicentre trial	VDBD (air)	40	Semi-ulcerated wound or skin ulcer with known aetiology for more than 60 days	Efficacy antimicrobial treatment and wound bed preparation of wounds older than 2 months	Reduction in bacterial load and accelerated wound healing. In addition, improved wound bed conditioning was also demonstrated.

of a plasma torch revealed a significant reduction of the bacterial load in chronic wounds.[166,172] Another case-control study showed a comparable antibacterial outcome after CAP treatment using an Ar PJ device when compared to an octenidine-treated group.[175]

While the primary endpoint of these feasibility studies was safety and reduction of bacterial load, a subsequent retrospective follow-up study conducted by Isbary and colleagues[166,172] provided insights on the potential of CAP to promote healing of chronic wounds.[173] A first mono-centric, two-armed, open, prospective, randomised and controlled pilot study using a DBD plasma source for the treatment of chronic leg venous ulcers enrolled 14 patients.[174] Fifty percent of the patients received conventional wound care, while the remaining half underwent CAP treatment in addition to standard wound care. In both groups, wound size reduction of approximately 50% was observed; however, the CAP-treated group exhibited a quicker and more substantial decrease in wound size after 3 weeks. Notably, one patient in the CAP-treated group achieved complete healing. Plasma treatment resulted in a significant reduction in lesional bacterial load (P=0.04, Wilcoxon signed-rank test). A more than 50% ulcer size reduction was noted in five of seven and four of seven patients in the standard and plasma groups, respectively. A greater size reduction occurred in the plasma group (plasma –5.3cm^2, standard of care (SOC) –3.4cm^2, P=0.42, log-rank test). The only ulcer that closed after 7 weeks received a plasma treatment originally. In total, two serious adverse events

(SAEs) and 77 adverse events (AEs) were observed, distributed equally among both groups (P=0.77 and P=1.0, Fisher's exact test).

Following these early studies on the antibacterial efficacy and safety, eight clinical RCTs with focus on the efficacy of CAP to enhance healing of chronic wounds were conducted.[176,178–182,184,187] One of the parameters to assess wound healing in these studies was wound size reduction. For example, significantly accelerated wound size reduction in the CAP treated group compared to the control group was observed by Chuangsuwanich and colleagues after treatment of pressure-induced ulcers using an Ar-based PJ.[176]

With regard to the various therapeutic effects of CAP, it is relevant to consider the 2020 study of Stratmann and colleagues in more detail (Table 3).[178] Results of this prospective, placebo-controlled, blinded, multicentre study showed a significantly more pronounced reduction in wound size, as well as reduced time to relevant wound area reduction with CAP treated subjects. Interestingly, no significant difference in the reduction of infection and microbial load was observed between CAP and placebo treated patients. This could suggest that the observed wound size reduction and time to closure changes might hold a direct causal relation to the CAP treatment received, independent of the (background) infection.[178] To further investigate this significant study finding, Hiller et al. analysed wound exudates of a sub-cohort of this study to evaluate the expression of FGF-2, VEGF-A, cytokines and matrix metalloproteinases (MMP) (Table 4).[190] These analyses revealed an increase of crucial growth factors like FGF-2, VEGF-A and interleukins, suggested to be important factors of CAP-mediated enhancement of granulation, vascularisation and renewed epithelialisation with diabetic foot morbidity.

Similar analyses were performed by Amini et al., who investigated cytokines and growth factors in the wound exudates retrieved during the earlier RCT by Mirpour et al., which considered the treatment of diabetic foot ulcers (DFU) with CAP (Table 3).[179,180] Where the clinical results of Mirpour and colleagues showed immediate antiseptic effects due to CAP treatment, the analyses of Amini et al. showed a significant reduction of IL-1, IL-8, tissue growth factors TGF-β, TNF-α, and INF-γ after 3 weeks (Table 4).[180] These results are, however, not corroborated by the later observations by Hiller et al. (Table 4).[190]

While some of the RCTs mentioned here focused on DFU, multiple other studies investigated the efficacy of CAP on pressure ulcers, therapy-refractory chronic wounds, chronic non-healing arterial or venous wounds on the lower leg, and infected or non-infected chronic wounds of different aetiology. However, at the time of writing, no study has been conducted which compares the efficacy of CAP for the treatment of wounds of different aetiology systematically. Therefore, it is still unclear whether wounds of a certain aetiology might be more prone to benefit from a CAP therapy than wounds of another aetiology.

As illustrated here, results indicating a beneficial efficacy of CAP in promoting healing of chronic wounds have been growing steadily, suggesting that CAP can reduce bacterial load, increase micro-circulation, accelerate wound closure, and reduce pain. Having said this, however, there are many differences between the various studies with respect to patient cohort, devices, treatment times and frequencies, and outcome measures. For example, most RCTs so far have been conducted with PJ devices, introducing the need for more non-jet plasma device studies. Considering the many differences between plasma sources, and the composition of the resulting plasma they produce, it is important that clinical efficacy is investigated for every device before routine clinical use can be suggested. Furthermore, it should be acknowledged that not every patient benefits from the addition of CAP to their standard wound care, despite the often observed significantly improved wound healing. Hence, more clinical research is required to identify patient groups that are most likely to benefit from CAP treatment. Potentially, parameters such as age, smoking, alcohol consumption, body mass index or wound aetiology could play a significant role in the clinical efficacy of CAP treatment.

4.2 Clinical evidence for the efficacy of CAP to promote healing of acute wounds

However, while a fair number of RCTs have shown beneficial effects of CAP on healing of chronic wounds, the evaluation of CAP to improve healing of acute wounds is still sparse (Table 5). In a series of case reports, sterile laser skin lesions were treated with an Ar-based PJ for either 10 seconds, three times 10 seconds, 30 seconds, or left untreated as control.[191] When wound healing was evaluated 6 and 12 months after treatment, the best outcomes were observed with subjects treated with the

Table 4: Cytokine and growth factor levels in wounds treated with CAP compared to control wounds.

Study	Study description	Protein	Change in CAP study group
Hiller et al. 2022[190]	Wound exudates of 13 wounds (11 participants) of the placebo group and 14 wounds (12 participants) of the CAP group from the KPWTRIAL[178] were collected over a period of 2 weeks and analysed for protein expression of growth factors, cytokines and MMP.	FGF-2 (growth factor)	Increased ($p<0.05$)
		VEGF-A (growth factor)	Increased ($p<0.05$)
		IL-1α (cytokine)	Increased ($p=0.07$)
		IL-8 (cytokine)	Increased ($p=0.10$)
		TNF-α (cytokine)	Increased ($p=0.10$)
		MMP-1	Unchanged
		MMP-2	Unchanged
		MMP-3	Unchanged
		MMP-8	Unchanged
		MMP-9	Unchanged
		MMP-13	Unchanged
Amini et al. 2020[180]	Wound discharge samples of patients included in the clinical study by Mirpour et al. 2020[179] were collected after 3 weeks of treatment and analysed for protein expression of inflammatory cytokines and growth factors.	IL-1 (cytokine)	Decreased ($p<0.001$)
		IL-8 (cytokine)	Decreased ($p<0.001$)
		INF-γ (cytokine)	Decreased ($p<0.001$)
		TNF-α (cytokine)	Decreased ($p<0.001$)
		TGF-β (growth factor)	Data not shown

Table 5: Overview of clinical studies assessing the efficacy of CAP in the treatment of acute wounds.

Study	Study subjects	Plasma technology	Main conclusions
Metelmann et al. 2012[198]	Five experimental case reports	PJ (Ar)	CAP stimulation of laser skin lesion recovery looks promising.
Metelmann et al. 2013[191]	Four laser lesions in five individuals	PJ (Ar)	CAP treatment seems to support the inflammation needed for tissue regeneration.
Heinlin et al. 2013[195]	Skin graft donor sites of 40 patients	Plasma Torch (Ar)	Donor site wound areas treated with CAP show significantly improved healing compared with placebo-treated areas.
Vandersee et al. 2014[194]	Six subjects with vacuum-generated wounds	PJ (Ar)	CAP leads to a significant more rapid area decline in comparison to no treatment, treatment with octenidine, and sequential treatment with CAP and octenidine.
Nishijima et al. 2019[193]	Four laser lesions in 12 volunteers	PJ (Ar)	No significant difference among all groups. Significant reduction in redness 1 day after treatment.
van Welzen et al. 2021[196]	Skin graft donor sites of 10 patients	VDBD (air)	CAP improves wound parameters such as deep tissue oxygen saturation, haemoglobin distribution, and tissue water distribution.
Matzkeit et al. 2021[197]	Skin graft donor sites of 20 healthy subjects	VDBD (air)	CAP increases cutaneous tissue oxygen saturation and capillary blood flow at the standardised acute wound healing model.

three times 10 seconds and single 30-second regimens. In another study, laser lesions were treated with CAP for 60 seconds and compared to ointment (betamethasone valerate and gentamicin sulfate), basic FGF, or no treatment.[192,193] Here, only a significant difference in the redness one day after treatment was observed. However, on day 3, 7, 14 and 28 after treatment, there was no difference between the various treated groups. A study by Vandersee and colleagues compared CAP treatment (Ar PJ) of vacuum-generated acute wounds with either

no treatment, octenidine treatment, or a combination of sequential CAP and octenidine treatment.[194] Their results showed that CAP treatment led to a more rapid area decline that was statistically significant in comparison to the other treatment groups. Morphologically, the authors reported that wound healing was found to initiate from the edges with the formation of dendritic structures consisting of keratinocytes.[194]

Some studies used skin graft donor sites to investigate the effects of CAP on acute wounds. In one of these studies, half of the skin graft donor wounds were treated with CAP, while the other half received placebo treatment.[195] When wound healing was assessed in a blinded fashion by independent experts, it showed that wound healing was improved significantly after CAP treatment using a plasma torch.[195] In a pilot study, the efficacy and safety of a novel VDBD plasma dress device has been explored for treatment of skin graft donor sites.[196] In this study, the course of wound healing was assessed using hyper-spectral imaging which revealed that CAP improves important wound healing parameters such as deep tissue oxygen saturation, haemoglobin distribution and tissue water distribution. Increased cutaneous tissue oxygen saturation and capillary blood flow have also been observed by Matzkeit and colleagues after treatment of skin graft donor sites with a VDBD device.[197] In all, these studies demonstrate a potential of CAP to improve wound healing parameters in acute wounds. There is, however, a clear need for larger RCTs to corroborate these early findings.

4.3 Treatment times and frequencies

To date, no standards for the clinical application of CAP in wound treatment have been established. Besides different plasma technologies and devices, there is a variety of parameters that differed between the clinical studies investigating the efficacy of CAP to promote healing of chronic wounds (Table 6). The studies varied, for example, by treatment time from around 1 second/cm^2 up to 7 minutes. In some studies, wounds were treated just once, while treatment was repeated up to five times a day or several times a week over a period of several weeks in other studies. Moelleken and colleagues compared a single treatment per week with three treatments per week, and observed that a single treatment is not inferior to the more frequent treatment.[181]

Preclinical studies indicate a treatment efficacy following the principle of hormesis.[97] Hormesis is a dose response phenomenon describing a stimulating effect of a treatment at low doses, and an inhibitory effect at higher doses. Against this background, a treatment schedule with few treatments per week, followed by longer treatment pause (2–3 weeks), is suggested for wound healing purposes.[199] For a sole antimicrobial and antiseptic treatment, a more frequent treatment, daily for 1 week, is suggested. However, due to the lack of studies comparing different treatment times and frequencies in a clinical setting, the optimal treatment modalities and the therapeutic window still need to be determined. Furthermore, it is also important to note that, because of the different plasma technologies, the optimal treatment modalities and therapeutic window will have to be determined for each device individually.

In general, it is recommended to follow the instructions by the manufacturer of the respective device. Nevertheless, more clinical studies are needed to gain further insights into the optimal treatment modalities. Furthermore, additional clinical observation will help identify profiles and characteristics of patients who will most likely benefit from CAP treatment. To date, clinical observations of CAP wound healing treatment results are accumulating steadily, as illustrated by systemic literature reviews.[200] General requirements for plasma sources in medicine are provided by the German Institute for Standardization (DIN SPEC 91315).[199] Due to continuous developments, this 2014 document is being revised currently, with an updated version expected to be published in German and English in 2024.

Table 6: Summary of plasma technologies and treatment modalities used in clinical studies for CAP treatment of chronic wounds.

Study	Plasma technology (feed gas)	Treatment time	Repetition and treatment period	Distance (slm)
Isbary et al. 2010[166]	Plasma Torch (Ar)	5 minutes	Daily (on average 7.86 treatments)	20mm (2.2)
Isbary et al. 2012[172]	Plasma Torch (Ar)	2 minutes	Daily (on average 11.75 treatments)	20mm (2.2)
Isbary et al. 2013[173]	Plasma Torch (Ar)	3–7 minutes	Daily (on average 7.90 treatments)	20mm (2.2)
Daeschlein et al. 2015[165]	PJ (Ar)	10 seconds/cm^2	Once	10–20mm (6.0)
Klebes et al. 2015[170]	PJ (Ar)	1 minute/cm^2	Once	7–8mm (5.0)
Brehmer et al. 2015[174]	VDBD (air)	45 second/cm^2	3× week for 8 weeks	2mm
Ulrich et al. 2015[175]	PJ (Ar)	1 minute/cm^2	3× week for 2 weeks	7–8mm (5.0)
Chuangsuwanich et al. 2016[176]	PJ (Ar)	1 minute/cm^2	1× week for 8 weeks	1–3mm
González-Mendoza et al. 2020[177]	DBD (He)	30 seconds/cm^2	Daily	5–10mm
Stratmann et al. 2020[178]	PJ (Ar)	30 seconds/cm^2	5× daily, then 3× every second day	Sterile spacers
Mirpour et al. 2020[179]	PJ (He)	5 minutes	3× week for 3 weeks	10mm
Amini et al. 2020[180]	PJ (He)	5 minutes	3× week for 3 weeks	10mm
Moelleken et al. 2020[181]	Plasma Torch (Ar)	2 minutes per application field	1× or 3× per week	(n/a)
Jensen et al. 2021[167]	VDBD (air)	90 seconds	3× with 10 minute pause	2mm
Samsavar et al. 2021[182]	PJ (He)	1 minute/cm^2	2× week for 6 weeks	3–5mm
Schleusser et al. 2022[183]	VDBD (air)	90 seconds	Once	2mm
Strohal et al. 2022[184]	PJ (Ar)	30 seconds/cm^2	3× week in first week, 2x week in second week, then once a week	Sterile spacers
Lagrand et al. 2023[186]	VDBD (air)	1 minute	Once daily for 10 days (week days only)	(n/a)
Abu Rached et al. 2023[187]	SDBD (air)	2 minute, 1.2 seconds/cm^2	3× week for 4 weeks	5mm, sterile spacer
Bakker et al. 2024[188]	VDBD (air)	2 minutes	1x or 2x per week for 12 weeks	(n/a)
Ligresti et al. 2024[189]	VDBD (air)	1 minute	1x per week for 30 days	(n/a)

5. Review of emerging CAP therapies: preclinical and clinical perspectives

CAP therapy is an emerging medical technology that has garnered considerable attention due to its potential applications in wound healing and various other medical conditions. Here, a comprehensive review of preclinical and clinical studies exploring the effects of CAP beyond current applications for wound healing is presented. While doing so, some future prospects of CAP therapy that might serve a valuable approach for promoting wound healing in the coming years are highlighted.

5.1 Exploring potential of CAP for wound healing

There are a significant number of papers showing positive effects or an absence of negative effects on cell cultures in vitro. Although these studies have no direct implications for the clinical use of CAP in wound care, they do provide valuable information for fine tuning of existing devices, the development of new CAP applications, and broadening the understanding of the different mechanisms of action; for example, short (<60 seconds) treatment with a CAP PJ of primary keratinocytes, immortalised keratinocytes (N/TERT1, HaCaT), and improved fibroblasts in vitro cell migration.[158,201,202] Similarly, short (60 seconds) treatments of primary keratinocytes with DBD enhanced migration, while longer treatments showed a pro-differentiation effect.[203] These results were demonstrated to be related to the concentration of H_2O_2 and were modulated by nitrite/nitrate.

As described earlier in this document (paragraph 3.1), animal studies showed CAP stimulates wound closure through increased cell proliferation and migration, and increased expression of collagens and alpha smooth muscle actin (αSMA). Furthermore, it also shifts the cytokine balance from a pro-inflammatory (mainly IL-1β, TNF-α and IL-6), and induces a switch in the macrophage subtype from a pro-inflammatory phenotype (M1) to a repair-promoting phenotype (M2). In addition, cellular antioxidant stress and deoxyribonucleic acid (DNA) damage repair are suggested to be enhanced by CAP.

In human trials, CAP was also shown to promote faster healing by disinfecting the wound and stimulating tissue regeneration, increasing tissue oxygen pressure, improving overall wound condition, and reducing infection rates compared to conventional therapies (Table 5).

Notably, CAP therapy has shown promising results in patients with hard-to-heal wounds, suggesting its potential as a non-invasive and effective alternative for challenging wound cases. Plasma can stimulate the micro-circulation of healthy skin beyond the treatment time,[164,167–169,204,205] which can improve the healing potential, especially of chronic wounds. The enhanced micro-circulation is probably mediated by plasma-produced NO[204] which easily penetrates the skin.[206,207] The potential role of plasma-generated NO in medicine is discussed in detail elsewhere.[208] NO is an important messenger and regulator of blood flow, immune response and wound healing, and NO can act as an antioxidant.

An increased micro-circulation might in part be due to heat produced by CAP. Heating skin to 42°C also stimulated blood flow,[209,210] while fast heating resulted in a higher endothelial activity.[210,211] Although referred to as cold or low temperature, CAP transiently increased the skin temperature [164,205,212] or resulted in a sensation of heat score of 0–5.[213,214] Furthermore, the effect of CAP on wound healing might in part be related to acidification by locally inducing nitrite and nitrate formation. Acidification of wounds by natural or artificial means plays a role in wound healing and the control of polymicrobial infections.[215]

It is important to note that changing the dosing or treatment regimens of CAP can affect the desired outcomes and/or side effects. Currently, several clinical trials are being conducted to investigate the application of CAP on various types of wounds, including chronic ulcers, burns and surgical wounds. A comprehensive overview of the status to date of these clinical trials can be found in Table 7.

Table 7: Overview of completed, but not yet published, ongoing or planned clinical trials for testing CAP devices.

Trials testing CAP for anti-tumour effects were not included.
ID: study identifier at clinicaltrials.gov (NCT) or onderzoekmetmensen.nl/en (NL); n: number of participants.
There were no studies found with CAP at https://euclinicaltrials.eu/ or https://www.clinicaltrialsregister.eu/

ID	Title of the study	Condition	CAP device	CAP type	n	Status
NCT04205942	Cold Plasma Therapy for Acceleration of Wound Healing in Diabetic Foot	Diabetic foot	Ar PJ	Ar jet	65	Active, not recruiting
NCT04965805	Cold Plasma Jet kINPen Med Versus Best Practice Wound Dressings	Chronic wounds of any origin or wound phase	kINPen Med	Ar jet	78	Completed
NCT03007264	Cold Plasma for Wound Treatment, Safety Study	Intact skin of volunteers	PLASOMA-prototype	FE-DBD	25	Completed
NCT04828304	PLASOMA Ultimate Safety & Efficacy Study	DFU, VLU, pressure ulcer, burn wound, skin graft, infected surgical wound, skin flap	PLASOMA	FE-DBD	100	Recruiting
NCT05855499	Plasma On Chronic Wounds for Epidermal Regeneration	Chronic ulcer of lower extremity	CPT®cube, CPT®patch	SDBD	167	Recruiting
NCT05894096	Air Cold Atmospheric Pressure Plasma Treatment for Acceleration of Venous Ulcer Healing	VLU	PJ	Ar jet	68	Recruiting
NCT02759900	Using a Cold Atmospheric Plasma Device to Treat Skin Disorders	Skin disorders	FPG10-01NM10, FID GmbH	FE-DBD	100	Recruiting
NCT05070754	Cold Atmospheric Plasma Device for Paediatric Molluscum and Verruca	Skin lesion	FPG10-01NM10, FID GmbH, Burbach, Germany	FE-DBD	67	Completed
NCT05592548	Rosacea Treatment Using Non-thermal (Cold) Atmospheric Plasma Device	Papulopustular rosacea	FPG10-01NM10, FID GmbH	FE-DBD	10–13	Suspended
NCT01662349	Safety Study of Plasma Treatment System to Treat Back Acne	*Acne vulgaris*	MOE Antimicrobial Plasma Treatment System	SDBD	8[12]	Terminated
NCT04886323	Effect of Cold Atmospheric Plasma on Malassezia Folliculitis	*Malassezia folliculitis*	Hand-held CAP, array with six copper needles	FE-DBD	90	Recruiting
NCT02658851	Cold Plasma for the Reduction of Lymphoceles Following PLND	Lymphoceles after PLND	Apyx Medical, Bovie Medical's J-Plasma® He plasma	He jet	100	Completed

ID	Title of the study	Condition	CAP device	CAP type	n	Status
NCT03072550	Early Feasibility Study to Evaluate the Efficacy and Safety of the RenewalNail™ Plasma Treatment System in Patients With Mild to Moderate Onychomycosis (Fungal Nail)	Fungal infected toenail	RenewalNail™	Air	30	Completed
NCT03216200	Early Feasibility Study to Evaluate the Efficacy of the RenewalNail™ Plasma Treatment System in Patients with Onychomycosis (Fungal Nail)	Patients With onychomycosis (fungal nail)	RenewalNail™	Air	5	Completed
NCT03286283	The Use of J-Plasma® for Dermal Resurfacing	Subjects with wrinkles	J-plasma (He PJ)	He jet	55	Completed
NCT04379752	Cold Plasma to Treat Hair Loss	Alopecia	He PJ	He jet	40	Recruiting

5.2 Blood coagulation

Blood coagulation is a critical process in the body's response to injury, playing a crucial role in preventing excessive bleeding. However, modern medical practices often require the ability to control blood coagulation rates. Various methods, like electric cauterisation and medications, have been employed, but may have undesirable side effects.

CAP has emerged as a promising tool for precisely managing blood coagulation with minimal adverse effects, and is studied as a means to control bleeding during surgery and promote haemostasis.[216] CAP likely promotes coagulation by enhancing the physiologic coagulation process through direct selective activation of fibrinogen, as well as through platelet activation, leading to aggregation and clotting[217,218] without affecting, for example, albumin, pH or Ca^{2+} concentration in blood. Furthermore, CAP's ability to control coagulation appears largely unaffected by the presence of anticoagulants, making it a versatile tool for surgical procedures and wound healing. It can be tailored to either accelerate or reduce coagulation, offering flexibility in medical application.

In the context of surgical procedures involving implantation and artificial implants, CAP's role is significant. Surface hydrophilicity of implants is a critical factor influencing clotting at the implant-bloodstream interface. CAP treatment can increase the hydrophilicity of implant surfaces, reducing clot formation and tissue adherence. Plasma treatment can significantly increase protein adsorption and cell adhesion of murine osteoblasts on different graft materials.[219] Moreover, CAP has shown the ability to induce natural coagulation around artificial implants, making it a valuable tool for managing clotting in these situations. The characteristics of CAP, such as power, feed gas and device type, can be adjusted to meet specific requirements for treatment. Overall, CAP could suggest a promising avenue for controlling blood coagulation, both for managing bleeding during surgeries and preventing excessive clotting around medical implants.

5.3 Modulation of implants

Treatment of implants with CAP allows for better integration into tissue or a lower risk of rejection,[31,219–221] although this seems more relevant for orthopaedic and dental surgery. The role of CAP application on biocompatibility and surface improvement in implantology is reviewed in an excellent review by Hui and colleagues.[222] CAP is capable of ameliorating surgical implants using various strategies of interface biotechnology, such as surface modification,[223–225] coating deposition,[226] and drug delivery, e.g. silver nano particles.[31] CAP modification dramatically increased surface pore size and wettability of a poly vinyl alcohol/poly lactic acid alcohol (PVA/PLA) core-shell scaffold, thereby increasing the loading capacity for medication.[227]

After transplantation of CAP-treated human acellular dermal matrix, fibroblast infiltration and proliferation was increased, indicating improved biocompatibility and bio-integration. CAP treatment significantly improved hydrophilicity, protein adsorption capacity, biocompatibility and bio-integration efficiency without compromising the structure of the human acellular dermal matrix.[509] Depending on the specific implant strategy, CAP enhancement of a surgical implant can be performed pre- or intra-operatively. For example, CAP can be used to maximise the bonding between adhesive and human teeth dentin or implant during a dental implantation surgery. Coating and drug loading are ideally prepared before the surgery, as the deposition process could be relatively time-consuming.

5.4 CAP indication extension, exploring other pathologies

The utilisation of CAP devices designed for skin resurfacing demands cautious handling due to the higher temperatures (>60°C) and long recovery periods for patients.[228–234] Ozone, which is produced by many CAP devices, can also be used as the single active component to treat atopic dermatitis.[235] In addition, CAP can modulate immune responses and alleviate symptoms. Currently, a He-based PJ is tested for the reduction of lymphoceles following pelvic lymph node dissection (see NCT02658851 in Table 7). Lymphoceles is lymphatic fluid that forms in a cavity in the body, typically as a result of surgery which disrupts the normal flow of lymphatic fluid. CAP can be used to dissect the lymph nodes and seal the lymphatic channels to prevent lymph leakage. This is slightly similar to the well-known plasma scalpel which has been used since the 1980s for the simultaneous division of tissue and coagulation of blood vessels.[236] These plasma scalpels do, however, exhibit higher temperatures to cauterise tissue, and are therefore not referred to as CAP.

CAP has been explored for various other therapies and treatments, such as skin rejuvenation,[237,238] actinic keratoses,[239] androgenetic alopecia,[240] herpes zoster,[241] warts,[242] dental applications,[243,244] bone regeneration,[245] rheumatism,[246] surgical site infections,[247] demodex mites[248] and psoriasis.[249,250] More recent fields of interest include ophthalmology[251,252] and cancer therapy.[253,254] An overview of recently completed or ongoing trials can be found in Table 7.

5.5 Cancer treatment

CAP has shown potential in cancer therapy by inducing apoptosis (programmed cell death) in cancer cells while sparing healthy cells. It is being researched as a potential complementary or alternative treatment to traditional cancer therapies, but is currently mainly used in in vitro and animal studies. Early-phase clinical trials have shown encouraging results, with CAP demonstrating potential anti-cancer effects in various tumour types and favourable safety profiles (Figure 2).

In 2015, a private company, US Medical Innovation (USMI), carried out a clinical trial on stage IV metastatic colon cancer at Baton Rouge General Medical Center in Baton Rouge, Louisiana, USA. CAP treatment was performed on the post-surgical tissue to kill potential residual cancer cells after a removal surgery. No relapse and progression of cancer occurred in patients.[254,255] In Germany, CAP treatment on 12 patients with advanced squamous cell carcinoma of the head and neck resulted in decontamination of infected cancer ulcerations, including a decreased request for pain medication and a reduction of typical fetid odour and microbial load.[256] In some cases, superficial partial remission of the tumour and even wound healing of the infected ulcerations have been observed.[256] Six patients with local advanced (pT4) squamous cell carcinoma of the oropharynx with open infected ulcerations were treated by an atmospheric pressure PJ (APPJ) in a cycle of three single applications within a week, each followed by an intermittence of another week.[257] CAP treatment noticeably improved the therapeutic effect of this locally advanced head and neck cancer (Figure 3). CAP treatment not only improved patients' social functions, but also caused a reduction in odour and pain medication requirements.

The mechanism by which CAP eliminates cancer cells is thought to be (in part) due to the higher expression of aquaporins and lower intracellular levels of lactase, which together result in a strong intracellular increase in ROS, leading to cell death (Figure 4).[258] CAP may also activate the immune system to attack tumorous tissue by reactive species or other factors. CAP could trigger cancer cells to emit signals known as damage-associated molecular patterns (DAMP), which may attract and stimulate local immune cells.[255] This suggests that CAP could possibly be used in combination with immunotherapy or chemotherapy to treat cancer.

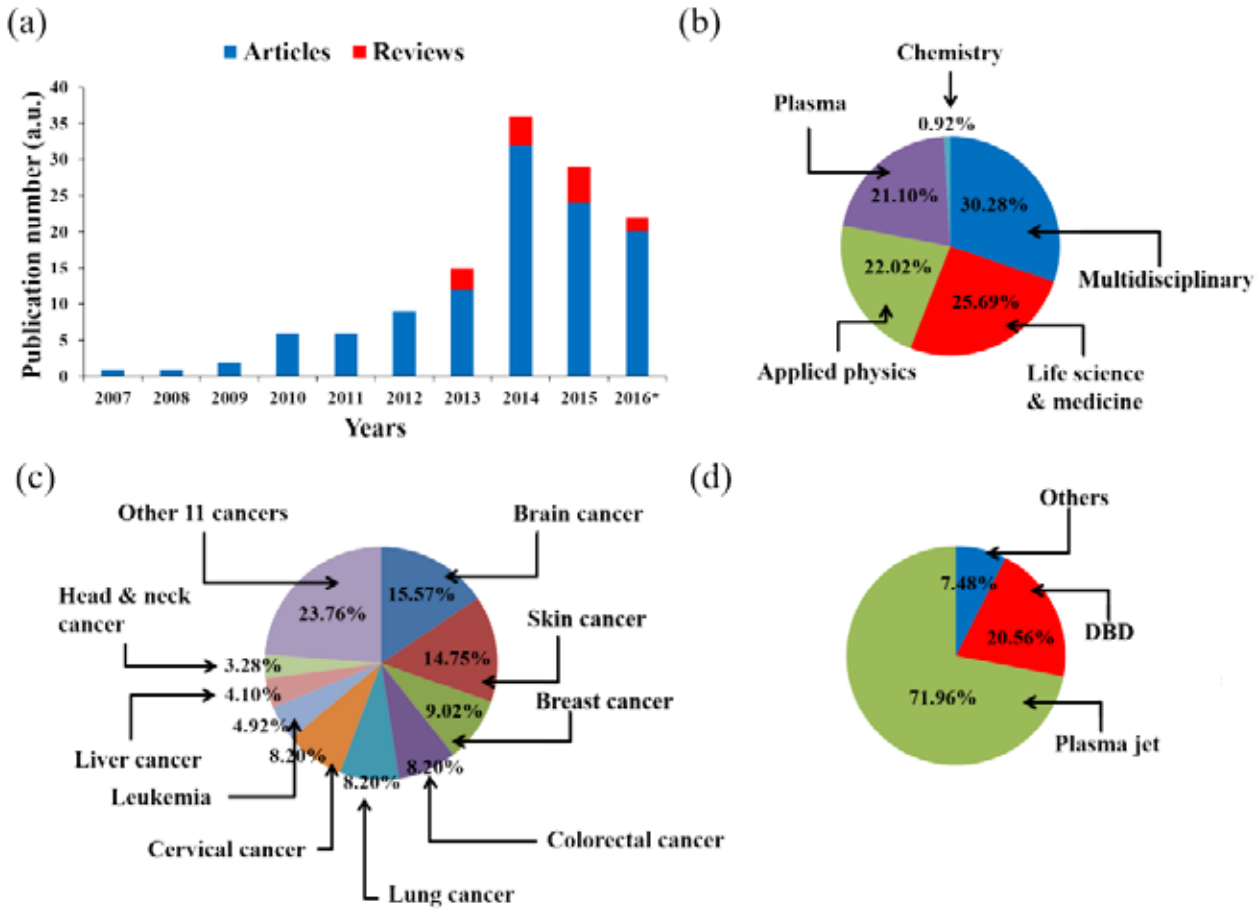

Figure 2: The research status of the application of CAP on cancer treatment by 2016.

*a) publication number; *) by the end of September; b) the journal type of articles; c) cancers in articles; d) plasma devices in articles. Reprinted with kind permission.* [258]

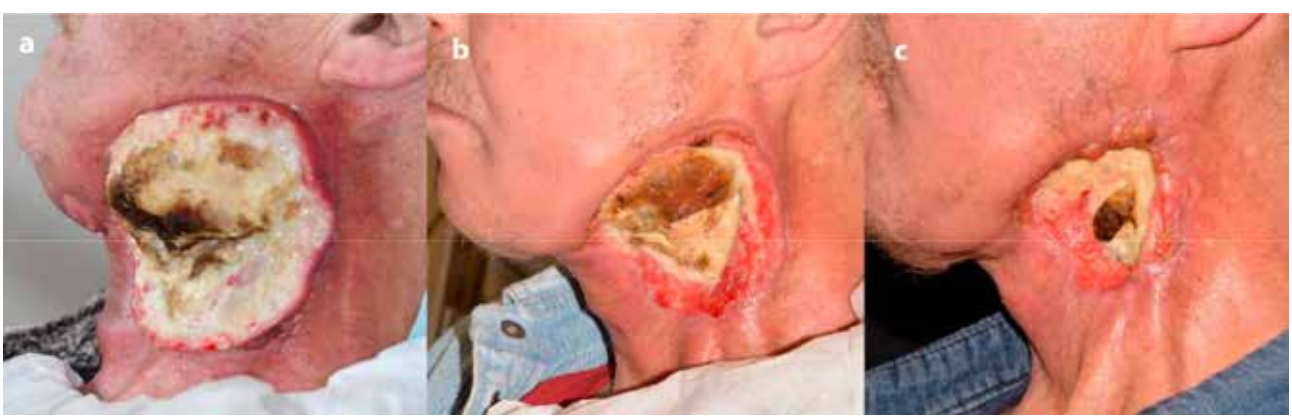

Figure 3: The clinical effect of CAP treatment on a patient with locally advanced head and neck cancer.

The patient's therapeutic effect was recorded in: (a) April 2016; (b) June 2016; and (c) August 2016.[257] *© Elsevier GmbH*

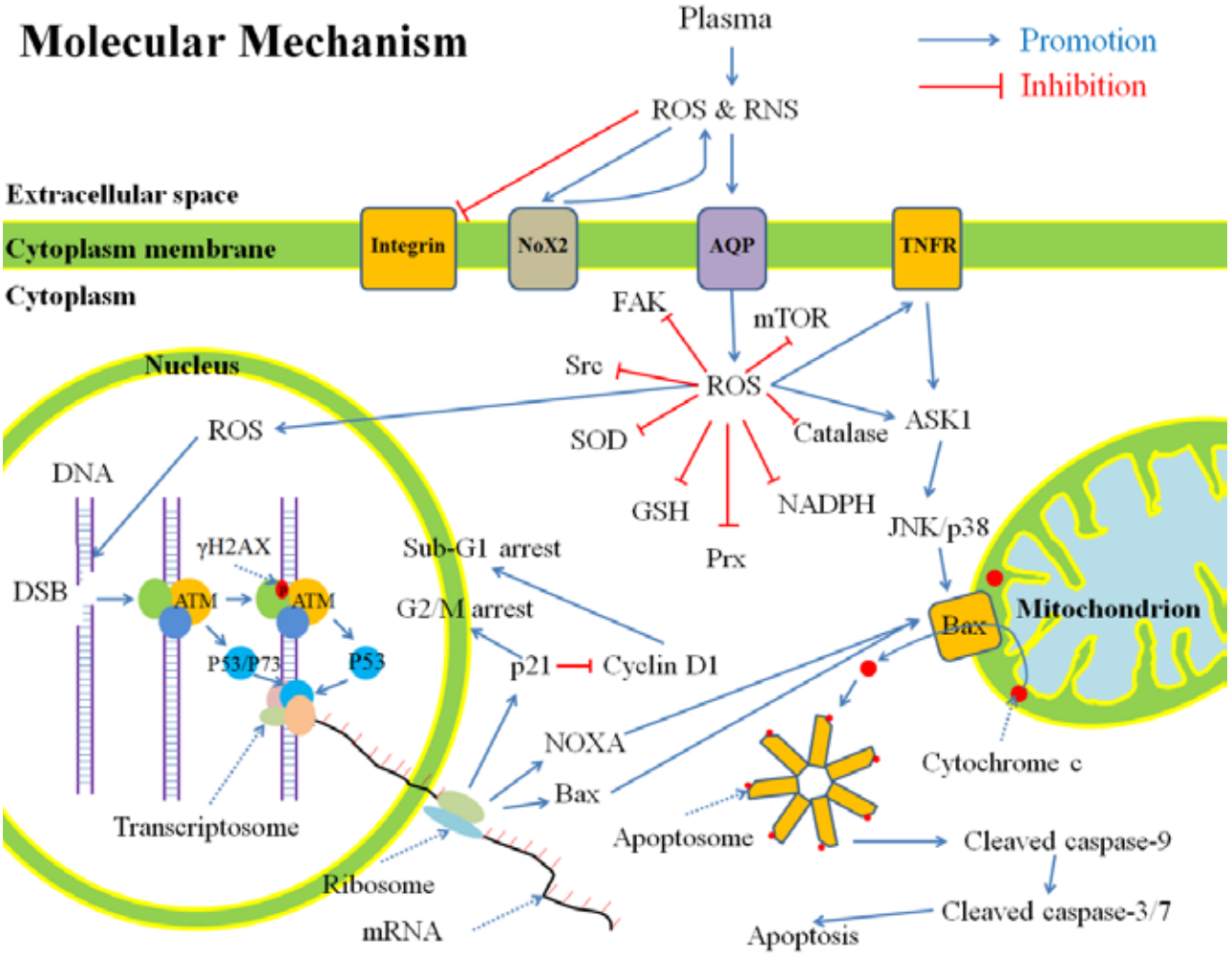

Figure 4: A general summary for the anti-cancer mechanism of CAP in vitro, based on publications.

The CAP-originated reactive species will cause a noticeable rise of intracellular ROS which weakens the intracellular antioxidant system and further causes serious DNA double-strand break (DSB). As a result, cell cycle arrest and apoptosis based on mitochondrion-pathway or tumour necrosis factor receptor-pathway occur.

ROS: reactive oxygen species; RNS: reactive nitrogen species; Nox: NADPH oxidases; AQP: aquaporins; TNFR: tumour necrosis factor receptor; FAK: focal adhesion kinase; Src: Src kinase; SOD: superoxide dismutase; GSH: glutathione; Prx: peroxiredoxin; NADPH: reduced nicotinamide adenine dinucleotide phosphate; mTOR: mechanistic target of rapamycin; DNA: deoxyribonucleic acid; DSB: double-strand break; ATM: ataxia telangiectasia mutated; mRNA: messenger ribonucleic acid; ASK: apoptosis signal-regulating kinase; JNK: c-Jun N-terminal kinase. Reprinted with kind permission.[258]

5.6 Other uses of CAP in a medical setting

5.6.1 Sterilisation and disinfection

Surface disinfection, especially in a medical setting, also plays an important role in infection prevention.[259] Careful cleaning and disinfection of environmental surfaces are essential elements of effective infection prevention programmes. However, traditional manual cleaning and disinfection practices in hospitals often prove to be suboptimal.[260] Disinfection of medical instruments, surfaces, rooms and vehicles can be done with CAP due to its ability to inactivate microorganisms and compatibility with all kinds of (sensitive) materials.[261] By enhancing clean practice environments through the antimicrobial properties of CAP, it is possible to reduce the risk of wound infections, and thus improve clinical outcomes.

During the COVID-19 pandemic, CAP was shown to be an inexpensive and sustainable technology for disinfection of face masks.[262] Based on this experience, the authors

suggest that therefore this disinfecting technology could also be applied to other objects and personal protective equipment used in hospitals. Furthermore, CAP can also be used for the sterilisation of implants, as reviewed in reference 222 and paragraph 5.3 of this EWMA document. One of the benefits of the utilisation of CAP is the speed of disinfection. For example, high log reductions were obtained after treating glass plates or silicone hands for 5–10 seconds with a SDBD device.[263] In addition, a period for neutralisation or cooling down after autoclaving is not required after CAP treatments. The management of biofilms on or in equipment by conventional methods or CAP is, however, more challenging.[264]

5.6.2 Air purification

Cold plasma devices are being explored to sanitise the air in hospital rooms and other healthcare facilities to help reduce the risk of healthcare-associated infections by eliminating airborne pathogens.[265,266] Plasma air treatment has, for example, shown to rapidly and effectively inactivate aerosol transmitted SARS-CoV-2 in rooms and therefore has great potential preventing the transmission of virus and infections.[267] In another study, CAP treatment significantly reduced bacterial counts in the air in hospital blood sampling rooms.[268]

5.7 Different sources for direct plasma delivery

At present, CAP devices that have been certified and approved for biomedical use in wound care are based on either PJ or DBD (Figure 1 and Table 8). In most studies with PJ, Ar gas is used.[200] Other CE-certified devices can be used during surgery for incisions (cauterise), or removal of tissues (ablation). These devices will not be discussed here, as these function at higher temperatures, and therefore cannot be regarded as CAP. Depending on demands, different delivery routes and plasma sources are explored such as biomedical device-assisted plasma delivery like in tubes,[269,270] for endoscopy,[271] large distances, or in patches[212] (paragraphs 5.8 and 5.9).

CAP device type, design and settings greatly affect outcomes and side effects. For example, distribution of reactive species on agarose gel or pig skin has proven to be strongly dependent on the discharge frequency.[272] When comparing two jet-based plasma devices in a side-by-side comparison, these differed in their effect on cell proliferation and migration.[53] The two devices did show similar effects on expression of collagen, cytokines and growth factors, activation of immune cells and improved wound healing.

In general, DBD devices can be used for larger surface areas than PJ. For the treatment of larger surfaces with jets, PJ arrays have been invented.[23,272–276] Electrosprays and plasma sprays can bring a wide range of potential biomedical applications, especially since the combination of plasma and aerosols is used in situations where direct contact needs to be avoided.[266] PJ can treat surfaces with irregular shapes because the reactive species are blown to the target area by the working gas. By adjusting the design of the plasma device, the plasma plume can be modified.[277–280] Nevertheless, the small size of the plasma treated area can lead to a low efficiency, or to long treatment times when treating large areas.[281,282] Furthermore, although PJ arrays can be used to treat larger areas, they are hindered by the complex interaction between the individual jets and the use of vast amounts of expensive gases.[276] Whether the direct plasma application of DBD is more effective than indirect plasma from jets has not been shown yet. As for the first DBD devices, these consisted of rigid plates which were not compatible with the curved human body. To anticipate this, various flexible variants of DBD have been developed and tested for the treatment of large areas with irregular surfaces.[212,281,283–289]

Floating electrode DBD (FE-DBD) is a DBD-based device which uses the treatment object, i.e. the patient, as a ground electrode,[290] and requires additional safety measures. For FE-DBD, the surface to treat should be flat to ensure a uniform discharge.[286,291,292] It can be regarded as a VDBD, for which the discharge occurs between the high voltage electrode and the grounded human body.[293–295] Contrary to FE-DBD, with SDBD, the plasma is produced on the surface of the dielectric[284,296–298] and does not require additional grounding to produce plasma. Extra grounding of the patient might be needed for safety regulations. Compared to SDBD, FE-DBD was more efficient in killing bacteria and produced less ozone, which is beneficial for biomedical applications.[212,265,299,300]

Thin films can be used as dielectric in DBD, which can be bent to treat different surface shapes, although bending can affect the discharge.[287] The development of a flexible plasma source that can be used to treat different shapes is of great significance for plasma medicine. Important issues are optimising the plasma dose, and minimising side effects in the wound and adjacent healthy tissues.[301] It might even

Table 8. Approved plasma devices for wound care and their indications for use.

Type of plasma	Indication	Device	Company
Jet	CE-marked silver applicator: non-ablative treatment, shallow and deep wrinkles, dilated veins, spider nevi, acne.	Jett plasma lift medical	Jett
Jet	CE-marked golden applicator: very deep wrinkles, acne, trauma and surgery scars, haemangiomas (benign mesenchymal blood vessel tumours) and minor venectasia, unwanted skin formations (fibromas), and stop minor capillary bleeding.	Jett plasma lift medical	Jett
Jet	Eliminate scars, smaller warts fibromas hemangiomas unwanted skin formations, angiectases, keratoses. Some indication (verruce vulgaris, condylomata accuminata, molluscum contagiosum) can be treated even inside natural body cavities.	Jett plasma medical II	Jett
Jet	FDA skin reduction, lifting, tightening and firming.	Jett Medical Plasma Pen	Jett
Jet	FDA: the treatment of moderate to severe wrinkles and rhytides, limited to patients with Fitzpatrick Skin Types I, II or III.	Renuvion Dermal Handpiece	Apyx medical
Jet	FDA: general use of cutting, coagulation, ablation of soft tissue during open and laparoscopic surgical procedures. For use in subcutaneous dermatological and aesthetic procedures to improve the appearance of lax (loose) skin in the neck and submental (under the chin) region.	Renuvion/J-Plasma handpieces	Apyx medical
Jet	CE-marked medical device for the treatment of acute and chronic wounds. It positively influences microcirculation as well as cell proliferation and has antimicrobial properties which makes it also suitable for wounds with local signs of infection and the therapy of pathogen-related skin diseases.	kINPen® MED	Neoplas med GmbH
Jet	To treat wounds, problematic wounds such as DFU, surgical site infections and dermatological conditions, actinic keratoses.	Steriplas	Adtec Healthcare
Jet	Coagulation.	maximum® beamer	KLS Martin group
SDBD	Wounds and treatment and prevention of viruses and bacteria.	Plasma Care	Terraplasma medical
SDBD	Treating wounds and any local infected tissue defects.	CPT®patch, CPT®cube	Coldplasmatech GmbH
FE-DBD	Chronic wounds, surgical wounds, skin diseases, exercise pain.	PlasmaDerm	PlasmaDerm
FE-DBD	CE marked device for chronic wounds.	PLASOMA	Plasmacure

be possible to use an adaptive plasma approach which is based on the ability to read the cellular response to CAP in real time and modify the composition and power of the plasma via a feedback mechanism.[302]

A fence-like plasma source might be used to generate a large area FE-DBD[288,303,304] which can even be produced as curved variants to match the treatment area.[288] These devices demonstrated good antibacterial effects and were operated at safe levels for human contact.[288,305] Furthermore, wearable plasma sources might be used to treat large areas with irregular shapes.[283,306]

Reactive species produced by jet or DBD devices penetrate various tissues or substrates (Table 9), although the depth of penetration is still insufficient and exact mechanisms are unknown. Plasma settings and target composition do affect penetration of different reactive species; hydrophobic RONS is translocated more easily across the stratum corneum than hydrophilic RONS.[307]

Several plasma devices are relatively large or are not integrated into an operation room easily. Ambulant and out-patient treatment requires transport of CAP equipment, nurses and/or patients. Therefore, it will be more practical,

Table 9: Penetration depth of RONS depends on CAP device and target surface.

Study	CAP device	Target	Maximum penetration depth
Duan et al., 2020[307]	He jet	Porcine ear stratum corneum in vitro	Through stratum corneum
Gaur et al., 2015[115]	He jet	PBS or BSA solution underneath gelatine gel	1mm
Nie et al., 2018[308]	He jet	Pig muscle in vitro	1.5mm
Omran et al., 2020[272]	He jet	Agarose gel	2mm
Fluhr et al., 2012[205]	Ar jet	Human stratum corneum (n=6)	24µm
Liu et al., 2022[282]	Ar jet	Agar gel, starch	0.8–6.6mm; 3.4–9mm
He et al., 2019[309]	Ar+O_2 jet	Gelatine gel, starch	250µm
Lee et al., 2019[289]	Flexible sDBD	Agarose gel	1.21mm
Heuer et al., 2015[204]	FE-DBD	Human skin in vitro	0.7mm
		Skin (microcirculation) (n=4)	8mm
Dobrynin et al., 2012[310]	FE-DBD	Chicken breast or rat skin in vitro, agarose gel	2–3mm

comfortable, cost-efficient and effective (in terms of compliance and frequency of utilisation) when small and affordable portable devices are available. If indeed so, this creates potential for at-home applications. Portable CAP devices have been used for skincare,[286,290] biomedical application,[280,311] to treat residual tumour cells in surgical cavities,[216] and as wearable fabrics.[285]

Although ozone is one of the reactive species involved in CAP-mediated bacterial killing,[292] production of high levels of ozone by CAP can limit its use by HCPs. Ozone can cause irritation of skin, eyes and mucous membranes of the respiratory tract, as well as drowsiness, dizziness, headache and fatigue. Safety limits for ozone inhalation vary between 100µg/m^3 (World Health Organization [WHO][312]) and 120µg/m^3 (European Union [EU][313]) for 8 hours per day. Tests with different types of CAP devices have been performed on cells or skin in vitro[14,74,80,204,212,314,315] or in vivo,[212,316] and the results on cellular activity, inflammation or DNA damage were found to be within safe limits.

5.8 Plasma-activated solutions for indirect plasma treatment

The range of CAP applications in the biomedical area is limited by two characteristics – permeability and manipulation. CAP only affects superficial layers, while deeper layers of tissue (muscle, bone and organs) are difficult or not reached by CAP (Table 9). As for manipulation, the direct use of CAP is limited depending on the presence and shape of the device, potentially in combination with the characteristics of the area to treat. These limitations were also encountered in other industries, especially environmental technology and agriculture, where plasma-activated solutions (PAS) have already been used in water treatment and food preservation.[317,318]

Solutions such as distilled water, saline solution or cell culture media can be activated by treating them with plasma.[261] After CAP treatment, PAS can be used for indirect plasma treatment. The benefits of this type of plasma treatment are highly controlled production parameters, higher plasma intensities or doses, limited safety issues, and availability of an off-the-shelf, easy-to-use and apply product for washing wounds or impregnating dressings. The shelf life of PAS solutions can range from hours to years depending on plasma dose and storage temperature,[319–323] and can be improved by changing the composition of the medium.[319]

The various ROS and RNS that are produced during the plasma–liquid interaction of the CAP treatment of PAS diffuse into the liquids. As a result, PAS contains the same reactive species as plasma produced during direct treatment with DBD or PJ.[296,324,325] The composition and efficacy of PAS for a specified condition can be improved by adjusting the production parameters. The chemistry of the reactive species in PAS and its application in the biomedical field was reviewed earlier by Zhou and colleagues.[296]

PAS has been found to be effective against bacteria,[322,326–328] fungi and viruses.[323,327,329–331] Initially, it was thought that acidification of the solution was the main antimicrobial factor of PAS. Studies, however, showed that both

acidity and RONS influenced the efficacy of PAS for the inactivation of microorganisms.[325,326,332–338] PAS can be used against bacterial biofilms[339,340] and to disinfect medical devices. Furthermore, PAS was found effective for irrigating peritoneal cavities in a rat acute peritonitis model.[341]

5.9 Plasma-activated hydrogels for indirect plasma treatment

In addition to solutions, plasma can also be used to activate hydrogels. Hydrogels exhibit excellent water storage and absorption properties, as well as observed favourable biocompatibility.[342–344] As such, they are used in a range of biomedical applications, including wound dressings,[345,346] and may be loaded with antimicrobial agents, antibiotics or metal nanomaterials.[347–349] Plasma-activated hydrogels (PAH) combine the properties of normal hydrogels with the capability to act as a carrier for reactive species.[350–352] Furthermore, PAH have demonstrated the ability to preserve and release plasma-derived reactive species over extended periods of time,[350,353] demonstrating long-term antimicrobial effects.[350] PAH also presents advantages where plasma-activated liquids (PTLs) may be diluted and/or washed away when treating patients.[354]

Hydrogels can be activated via direct or indirect plasma treatment. Indirect activation of PAH involves either plasma treatment of the aqueous polymeric solution or use of plasma-activated water (PAW) instead of non-treated water prior to cross-linking, resulting in a hydrogel encapsulating plasma-generated RONS.[351,354] Direct activation of PAH, however, involves plasma activation after cross-linking and hydrogel formation.[351] The chemistry of the polymers used for hydrogels will affect the species and concentration of RONS generated.[354] PAH have been formed from a wide range of polymers, including alginate,[352] gelatine,[355] methylcellulose,[356] hydroxyethyl cellulose,[351] carbomer 940,[351] ammonium acryloyldimethy taurate/VP copolymers,[351] polyethyleneoxyde based copolymers,[357] and polyethyleneglycol–polylactide copolymers.[353]

Hydrogels also provide the ability to screen out short-lived and highly reactive species from direct plasma when placed between the plasma source and target. These include hydroxyl radicals (•OH) which have been linked to biological effects including phagocytosis, apoptosis and DNA damage.[358] Longer lived species, such as H_2O_2, are still delivered through the hydrogel screen.[358]

PAH can also be used as drug delivery vehicles. Injectable hydrogels, treated with plasma prior to injection and cross-linking (in-situ polymerisation), have been proposed for drug delivery treatment in cancers.[354] Hydrogels loaded with therapeutics such as antimicrobials, prior to plasma activation, have also been demonstrated, utilising a system whereby cationic drugs are loaded into sodium polyacrylate particles (PAA) contained within a secondary polymer matrix (e.g. PVA). Subsequent application of CAP releases the drug from the hydrogel "on demand".[359] To minimise systemic toxicity and improve tissue penetration of CAP, an injectable pluronic hydrogel was used as a delivery method. ROS and RNS in CAP were effectively preserved in the hydrogel and remained efficacious in inducing immunogenic cancer cell death after intratumoral injection.[359]

6. Safety aspects of plasma technology

6.1 Safety definition

Novel clinical and medical technologies and therapies need to be effective and safe. In 2013, two devices, one DBD operated in ambient air, and one atmospheric pressure Ar PJ, were approved for treating non-healing wounds and infected skin and appendices. Several other devices followed.[360]

When reviewing the topic of safety in plasma science, several things are important to note. First, clinical plasma devices are a class of therapeutic technologies, and not a single, uniform type of therapy. Instead, devices differ in geometries, discharge mechanisms, power and electric parameters, reactive species mixtures generated, and application. Therefore, safety assessment needs to be performed for each medical plasma device separately, not only from an academic point of view, but also – at least in Europe – under the requirements of Medical Device Regulation (MDR). It is important to note that there are a number of viewpoints on the safety of the application of a medical product. This includes regulatory aspects on the one hand, and caregiver and patient safety during medical product application on the other. In addition, molecular safety aspects should be considered. These may not affect overall health in general, but may be indicators that such effects may occur later, e.g. the "potentially carcinogenic" classification defined by the European Environment Agency (EEA) for suspected carcinogens. From a practical point of view, it is essential to acknowledge that 'safe' does not necessarily mean without side effects or risks. Safe also can also mean that potential side effects and risks are considered acceptable when weighed against the benefits of the specific therapy. Furthermore, such safety assessments are always relative and not absolute, as they are dependent on the boundary conditions of the specific applications defining the margins within a safe operation, e.g. maximum allowed plasma exposure times per area unit. It is therefore important to note that this chapter does not contain details on all aspects of all approved plasma devices (or their prototypes), since this chapter's information relies on peer-reviewed international journal publications.

6.2 Physical safety of plasma devices

Medical gas plasma devices are systems based on physical modalities generated through electricity. Accordingly, several features of plasma need to be considered. Importantly, all items refer to the noted correct application of the device. If, for example, a PJ is held too close to its target, or a plasma DBD device is applied much longer than indicated, the plasma application can leave the indicated safety margins. The first to note is the temperature. Per definition, cold plasmas need to be cold, which is roughly defined as not being significantly above body temperature or 40°C, a temperature at which protein denaturation can set in. Accordingly, it has been shown for several plasma devices that their temperatures are within this margin.[21,53] The second noteworthy item is UV radiation. According to the International Commission on Non-Ionizing Radiation Protection (ICNIRP), the maximum effective (weighted) exposure of 30 J/m^2 per day (8h; λ=180–400nm) should not be exceeded. This is the case for several plasma sources.[21,361] The third item is the production of toxic gases, such as higher concentrations of ozone and nitrogen oxides. Analyses have shown gas levels below toxic thresholds.[14,360] The fourth item is electrical safety, e.g. patient leakage current, which should be below 100µA, as is the case for all certified plasma devices in medicine.[362–364] More on safety by design of plasma devices can also be found in Appendix II.

6.3 Safety of plasma application in humans

The following section reports on findings related to safety applications of CAP exposure to intact and wounded human skin, excluding tumour wounds and other dermatology disorders. For the latter, CAP treatment is not recommended to date, based on medical guidelines.

Most studies on patient safety and tolerability of CAP exposure were carried out for an Ar PJ approved as medical product.[21] For its prototype, investigated prior to approval as medical product, treatments of the intact skin of fingertips of four human subjects at bactericidal exposure times were tolerated well. Specifically, the self-reported scores

for paraesthesia, pain and heat on a scale from 0–10 for CAP exposure times 2–4 times longer (150–240 seconds) than bactericidal dosages (60 seconds) ranged from 0.5 to 2.0 for all three parameters investigated.[165] For the Ar PJ, a complementary study in seven subjects found the treatment to be safe to skin physiology under clinical conditions in terms of a modest to absent change in trans-epidermal water loss and beta-carotenoid levels.[205] In a third Ar PJ study in ten other volunteers, it was demonstrated by laser-scanning microscopy that the treatment of intact human skin does not change the properties of the upper skin layer.[365] In addition, Ar PJ treatment of experimental laser wounds in five human subjects forearms promoted wound healing,[98] while not inducing scar formation if investigated in a 1-year follow-up study,[191] or a 5-year follow-up study in the same patients.[366] None of them reported on any acute or long-term AEs. In the case of experimentally generated low pressure induced wounds in intact skin of six healthy volunteers, Ar PJ treatment was overall well tolerated, and induced only mild burning sensations in some subjects, which lasted only as long as the duration of the exposition (60 seconds).[194] In four patients subjected to radial forearm free flap donor site surgery, creating acute wounds, Ar PJ exposure could be performed without occurrence of undesired AEs.[367] With regard to Ar PJ treatment of chronic wounds, 37 patients, self-reporting on a numerical scale, reported less pain during CAP exposure compared to the application of standard wound antiseptics.[170] In another clinical trial, 16 patients received either Ar PJ or antiseptic treatments, of which both treatments were tolerated well.[175] Finally, in a prospective, randomised, placebo-controlled, patient-blinded clinical trial, Ar PJ treatments of chronic wounds of 29 patients were well tolerated.[178] No SAEs related to the study intervention were described in Ar plasma exposure or the placebo group. The latter received a mock treatment with an Ar PJ being moved over the wound while its electric power was switched off. Other expected AEs, like for example scar formation, skin irritation and bleeding, were distributed evenly between both treatment groups. With time, all wounds healed, but Ar PJ-treated wounds healed significantly faster.[178]

Table 10 lists observed AEs and SAEs and, if available, their frequency in wound healing studies involving plasma, based on different aetiologies such as chronic ulcers, acute skin graft wounds, burn wounds and DFU. It has not been investigated whether the events listed are related causally to the plasma source used in the respective study. To date, there is no clinical study that investigated more than one plasma device. Due to the lack of a generally definable dosing concept, differences between plasma devices and their safety profiles may not be directly comparable. However, for each plasma device on the European market, at least one clinical study or case collection has reported its safety profile.

Specifically, regarding plasma sources approved for wound treatments in Europe for ambient air-operated or Ar gas-operated DBD, several studies were performed to assess the safety of treatment in human probands and patients suffering from chronic wounds. Acute wound treatment with an Ar-driven DBD was well tolerated in 34 patients.[195] Exposure with the same regulatory approved plasma source, or its investigative precursor prototype, was also well tolerated in a total of 130 patients suffering from chronic wounds described in three reports.[166,172,173] A single centre, two-armed, open, prospective RCT in 14 patients suffering from chronic wounds reported that treatments with another approved air-operated DBD were well tolerated.[174] Six patients indicated some pain and mild hyperthermia. One SAE was reported, which was unrelated to the treatment.

Recently, the first regulatory approved plasma plaster patch air-operated DBD was investigated in a randomised, multicentre clinical trial in 47 chronic wound patients, where the control group received standard wound therapy according to the current guideline without CAP therapy. In an interim analysis, no SAEs were reported in both the CAP treatment and standard wound treatment group.[187] Some patients perceive CAP treatment as tickle or mild burning during treatment, but not afterwards. Available studies seem to indicate an evidence-based favourable safety profile with regard to the exposure to, and the treatment of, human skin and acute and chronic wounds.

In September 2023, the German Federal Joint Committee (G-BA, Gemeinsamer Bundesausschuss), an institute independent of the German Ministry of Health authorised to make legally binding regulations and directives for German healthcare providers and payers, commissioned the manufacturers of CAP devices to supply clinical data in order to secure future treatment reimbursement. In response, three German manufacturers of cold plasma technologies have joined forces, and commissioned

Table 10: Overview of AE/SAE reported in clinical studies involving plasma treatment.

Reference	Patient number	Safety / tolerability statement	AE / SAE (in treated patients)
Shekhter et al., 1998[368]	68 (case collection)	None	None reported
Isbary et al., 2010[166]	36 (randomised)	"No side-effects occurred and the treatment was well-tolerated"	None reported
Isbary et al., 2012[172]	24 (randomised)	"No side effects occurred and the treatment was well tolerated"	None reported
Metelmann et al., 2012[198]	5 (volunteers)	None	None reported
Heinlin et al., 2013[195]	39 (randomised)	"Mild reversible adverse events…such as sensations of minor burning or discomfort, prickling, and warmness".	13 / 0
Isbary et al., 2013[173]	70 (randomised)	"No side effects were reported and the treatment was well tolerated in all cases".	None reported
Vandersee et al., 2014[194]	6 (volunteers)	"Plasma […] proved to induce only mild burning sensations in some subjects, which was well tolerated and lasted only as long as the duration of the exposition".	None reported
Brehmer et al., 2015[174]	14 (randomised)	"This SAE was labelled unrelated to the plasma treatment". "Frequently reported AEs in both arms were ulcer pain and problems with the skin adjacent to the wound (stasis dermatitis, maceration)".	6 / 1
Klebes et al., 2015[170]	34 (case collection)	"All antiseptic procedures were associated with only minimal pain as measured by numeric scales. Interestingly, TTP [plasma] alone was better tolerated than ODC [Octenisept] concerning the level of pain".	None reported
Ulrich et al., 2015[175]	16 (case collection)	"Plasma…exerts an antimicrobial effect in chronic wounds without any side-effects".	None reported
Chuangsuwanich et al., 2016[176]	50 (randomised)	"No side effects were reported and the treatment was well tolerated in all cases".	None reported
Betancourt-Angeles et al., 2018[369]	1 (case report)	"The patient reports having no discomfort except in the crusts that have a little itch, in particular, the crust area was not modified and remained in the 9cm^2. Also, there is a process of re-epithelialisation of both wounds and no longer bacterial presence".	None reported
Hartwig et al., 2017[367]	4 (case collection)	"No undesirable side effects were observed and no inflammation or infection occurred".	None reported
Hartwig et al., 2017[370]	6 (case collection)	"No undesirable side effects were observed, and no inflammation or infection occurred after cold plasma initiation".	None reported
Naderi et al., 2017[371]	4 (case collection)	None	None reported
López-Callejas et al., 2018[372]	1 (case report)	"The patient reported little pain while having an infection, when this was eliminated, the patient reported only pain when the cleaning and debridement were performed, which was attenuated significantly as soon as the [plasma] treatment was applied".	None reported
Pekshev et al., 2018[373]	1 (case report)	None	None reported
González-Mendoza et al., 2019[177]	32 (case collection)	"Plasma…significantly accelerated the wound healing, this being a painless, effective, and mainly safe procedure".	None reported

Reference	Patient number	Safety / tolerability statement	AE / SAE (in treated patients)
Nishijima et al., 2019[193]	12 (volunteers)	"Plasma treatment exhibited several benefits: anti-inflammatory activity equivalent to that of steroid ointment, capability for continuous irradiation for each patient, and no side effects".	None reported
Gao et al., 2019[374]	7 (case collection)	"There was no significant adverse effect observed in the seven patients after CAP treatment".	None reported
Amini et al., 2020[180]	44 (randomised)	"Cold atmospheric helium plasma treatment is safe and painless new technique to decrease bacterial load for chronic infected wounds".	None reported
Mirpour et al., 2020[179]	44 (randomised)	"No specific harm was reported in the study".	None reported
Stratmann et al., 2020[178]	45 (randomised, multicentre)	"No therapy-related adverse events occurred during therapy". "All severe unexpected adverse events were qualified as not being related to study therapy or procedures and resolved shortly after occurrence".	7 / 1
Van Welzen et al., 2021[196]	10 (controlled pilot study)	"CAP treatment was well tolerated. Pain decreased in both wound areas during the study treatment and was significantly lower in the CAP-treated areas […] consistent with previous studies, in which CAP was able to reduce pain in skin infections".	None reported
Boekema et al., 2021[212]	25 (healthy volunteers)	"Intact skin of 25 healthy volunteers was treated with CAP for 3× 20" to determine safety. Although participants reported moderate pain scores (numerical rating scale 3.3), all volunteers considered the procedure to be acceptable. SAEs did not occur. CAP treatment resulted in a temporarily increased local skin temperature (≈3.4°C) and increased erythema. Lowering the plasma power resulted in a significantly lower erythema increase".	Transient AEs (grade 1) were reported: warmth sensation (all subjects), itching until one day after treatment (one subject in group A). SAEs did not occur.
Strohal et al., 2022[184]	78 (multicentre, randomised, open label)	"To determine local tolerability, presence or absence of erythema, maceration, blisters and congestion of exudate were assessed. While in the CAP-jet group none of the disorders mentioned occurred (n=0), in the BP group three patients suffered from erythema; furthermore, wounds of three patients showed signs of maceration and in further two cases other disorders were detected [...]. In both groups, no AE as well as SAE occurred during the study period".	None reported
Abu Rached et al., 2023[187]	47 (randomised, multicentre)	"Our results demonstrate that cold plasma therapy (CPT) can effectively reduce wound pain, even reducing the pain score to zero on the numeric rating scale, which is a significant relief for patients".	None reported

Reference	Patient number	Safety / tolerability statement	AE / SAE (in treated patients)
Lagrand et al., 2023[186]	20 (single arm, open label)	"Most adverse events were mild (grade 1) and transient. No serious adverse device effect (SADEs) occurred. Three patients (15%) experienced an infectious SAE at the site of application within one month of treatment. In those cases, the infection occurred at the site of the plasma application, but it was unlikely to have been caused directly by the device or the study procedure. SAEs unrelated to the device included pneumonia in one patient; another patient underwent a toe amputation on the contralateral foot, and one patient developed a soft tissue infection of the ipsilateral proximal part of the lower leg".	55% of subjects reported transient mild grade 1 AEs
Bakker et al., 2023[188]	46 (randomised, open label)	Three SAEs were reported: in the control group, one patient was admitted to hospital (reason unknown) and another to a care institute (after a fall), and in the 2x direct-CAP group, one patient died due to an aneurysm. All three SAEs were not anticipated and not related to direct-CAP treatment.	None reported

a scientific institute to perform a randomised, double-blinded, multicentre trial with a planned population of 700 patients. This trial will generate the first data on the direct comparability of the efficacy and safety of different plasma devices.[375]

6.4 Safety from the molecular and preclinical perspective

Preclinical and molecular studies are important to analyse the potential risks of new therapeutics, especially regarding their potential carcinogenic action. There are several common assays, of which two are the Organization for Economic Co-operation and Development (OECD) 471 Bacterial Reverse Mutation Test, also known as AMES test, and the OECD 490e In Vitro Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene, performed in mouse lymphoma cells. There are no publications available with results of either of these commonly used assays with any of the certified medical plasma devices. However, results with two other OECD assays with CAP devices do exist: the cytokinesis-block micronucleus cytome assay In Vitro Mammalian Cell Micronucleus Test (OECD 487) for testing chemicals, also referred to as cytokinesis-block micronucleus or MN test;[376] and the second assay which is the hypoxanthine phosphoribosyl transferase (HPRT) test (OECD 476), validated for industry purposes. The studies performed with these two assays are summarised in Table 11. In these studies, where CAP was applied to the cells directly, without any time delays using additional carrier solutions, none showed evidence of plasma-induced genotoxicity in the in vitro laboratory cell culture models.

In addition to these in vitro studies, three critical in vivo publications are available. The first is a quantitative

Table 11: Overview of OECD 476 and 487 assays performed with CAP devices.

CAP source type	OECD genotoxicity assay employed	Finding	Reference
DBD operated with Ar	HPRT test	No genotoxicity	377
DBD operated in air	HPRT test	No genotoxicity	361
DBD operated in air	HPRT test	No genotoxicity	212
Atmospheric pressure Ar and neon PJ	MN test	No genotoxicity	378
Atmospheric pressure Ar PJ	MN test	No genotoxicity	379
Atmospheric pressure Ar PJ	HPRT test, MN test	No genotoxicity	380
Atmospheric pressure Ar PJ	MN test	No genotoxicity	381

genotoxicity evaluation by MN test in living chicken embryos. In a comparison of exposure times and conditions, i.e. liquid-covered versus dry tissue, no genotoxicity could be shown for a clinically compliant atmospheric pressure Ar PJ.[382] The second study investigated repetitive Ar PJ treatments (seven applications in 2 weeks) in experimental wounds in mice, which were analysed after 12 months.[144] When investigated with PET-CT, MRI and histopathological analysis, none of the CAP-treated animals showed any tumour growth, abnormal tissue proliferation or fibrosis. The third animal study investigated mucosal wounds treated with repeated Ar PJ exposure, i.e. once a month for 12 months at two different exposure times.[161] None of the 43 mice showed any tissue abnormality or oncological formation. Additional performed mouse studies also observed no malignant or pre-malignant tissue formation in intact skin after long-term Ar PJ application for 3 months.[383]

7. Regulatory submission and approval: a critical step to reach patient bedsides

7.1 Regulators: addressing high wound healing unmet need

There are many critical milestones on the development path of an innovative and effective therapeutic solution. However, few of these milestones are as significant as the process of obtaining regulatory approval. After all, when a medicinal product or medical device does not finalise its journey from bench to bedside by gaining market access, all previous product development investments have been futile. That this simple fact also applies to potentially new wound healing solutions was confirmed again recently by the US Food and Drug Administration (FDA). In a perspective publication by Verma et al., the FDA shares that it understands that innovative product development is critical for addressing the significantly increasing wound healing pathology prevalence and incidence.[384] Due to its high unmet medical need, and relatively limited research and funding, the FDA identifies non-healing chronic wounds as an area of priority, and therefore intends to help advance product development for non-healing chronic wounds for the ultimate betterment of patients.

Although the recognition and proffered help by the FDA are certainly to be appreciated, it will be interesting to learn what this help might mean for the regulatory obligations and requirements of new innovative wound healing solutions specifically. For example, Verma et al. confirm that "Regarding challenges in clinical trial execution, difficulties with patient enrolment, heterogeneous study designs with varying standard of care protocols and study populations, and difficulty achieving the most commonly utilized primary efficacy endpoint of complete wound healing result in high rates of trial failure, singly as well as collectively further impeding the development of innovative products".[385–389] It is therefore not surprising that, according to the authors, "FDA understands that there is external stakeholder interest to provide updated guidance to the 2006 'Guidance for Industry: Chronic Cutaneous Ulcer and Burn Wounds-Developing Products for Treatment', especially since no new chemical entities have received FDA efficacy approval for the treatment of chronic wounds since decades, in part due to an inability to reach the FDA accepted end point of 'complete wound closure'".[385–387,390] And although the FDA reminds stakeholders that "guidance documents are a set of recommendations. An alternate approach other than one proposed in guidance documents may be used", the frequent inability to reach the full wound closure end point within a clinical study timeframe, and the repudiation of alternative healing end points for regulatory approval, might demonstrate the intricate and delicate nature of this last critical product development step in everyday practice.[384,385]

7.2 Determining mechanism of action and dose response

Although European regulators do not seem to share the FDA wound care focussed insight, attention and urgency, the base principles of regulatory evaluation of new wound healing therapies are surprisingly similar between the USA and Europe. Per definition, a regulatory evaluation classifies a new therapeutic solution into predefined categories, summarised crudely as a determination of what it is, what it does, and how it does it. The determination of the mechanism of action is one of the first basic elements of this process.

In 1857, Johann Heinrich Wilhelm Geissler, a German physicist and glassblower, produced a sealed glass tube filled with a "noble" gas and two electrodes at each end; this was the great-grandfather of the neon light tube and modern-day energy saving compact fluorescent lamp (CFL).[391] Geissler did so on the request of Julius Plücker, professor of physics at the University of Bonn, who one year later published their observations with the 'Geissler tube'. Inspired by their findings, Sir William Crookes presented his findings on "streams of radiant matter" after applying high voltages to the poles of "highly exhausted glass tubes" in 1897.[392] Since then, "radiant matter", dubbed plasma in 1928 by Irving Langmuir, has found its way to several medical applications (Chapters 4 and 5). Due to its complexity and

multifaceted nature, the exact mechanism of action of CAP in wound healing is unknown, and therefore remains an ongoing subject for further study. As also described in detail in Appendix I of this EWMA document, plasma is an ionised gas composed of charged particles, electronically excited atoms and molecules, radicals and UV photons.[99] The type of energy input, voltage or discharge power, gas component, gas pressure, and radiation type of electric field determine the exact composition and properties of produced plasma.[393] By varying these plasma generation parameters, a low-temperature state (<40˚C) therapeutic plasma can be produced, with temperature values of ions and neutral particles much lower than those of electrons.[8] During discharge, ROS, RNS, RONS, charged particles, and electromagnetic and UV radiation are formed, which can influence biological systems.[394]

As was also noted earlier in this document, it is still a significant subject of research to determine how the RONS are delivered into biological targets, and what the exact interaction with various components of a tissue is, especially when considering the lifetime, diffusion rate and major physical barriers to traverse.[395] Despite this, the diffusion and delivery of plasma-generated RONS, or the stimulation of intracellular RONS generating mechanisms as a result of cold plasma treatment, have been suggested to regulate cell activity in both intensity- and time-dependent manners, at least indicating some kind of dose-response relationship.[102,396] Appropriate low levels of ROS produced by cold plasma treatment at a suitable intensity and time have been reported to enhance directly the proliferation and migration of skin-related cells, extracellular matrix (ECM) protein synthesis, cytoskeletal architecture, cytokine and growth factor production, and changes of junctional proteins between the cells, and to increase wound healing enhancing angiogenesis and (micro) circulation (FGF, angiopoietin, VEGF, TGF) (Figures 5 & 6).[57,58,150,168,202,397–401]

High levels of ROS, however, produced at a high intensity or over a long period of time, are seen to inhibit cell proliferation of, for example, endothelial cells, keratinocytes and fibroblasts.[57,102,183,200,402,403] Furthermore, CAP-induced immunomodulation was observed, showing that the time to maximum ROS production in human polymorphonuclear

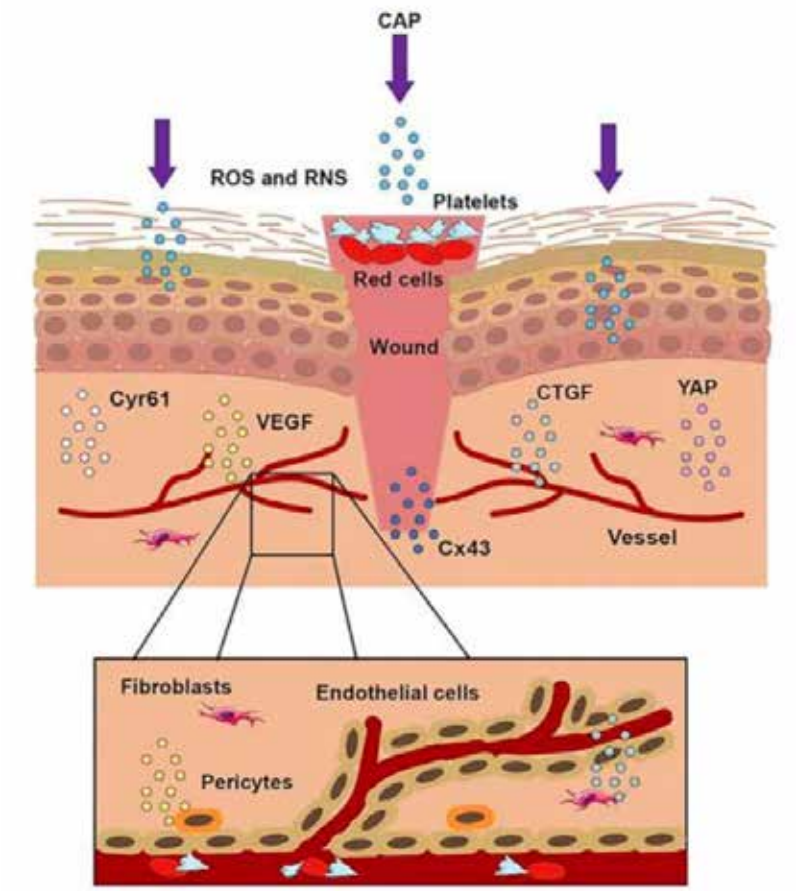

Figure 5: The treatment of CAP on a wound.

When the skin was injured, the first step was to form a blood scab to protect the wound. CAP could accomplish wound healing through short-lived and long-lived ROS and RNS. CAP could promote the formation of new blood vessels, strengthen the release of connective tissue growth factor (CTGF) and vascular endothelial growth factor (VEGF), activate the yes-associated protein (YAP) pathway, and upregulate the expression of Connexin 43 (Cx43) and Cysteine-rich angiogenic inducer 61 (Cyr61). Reprinted with kind permission [58]

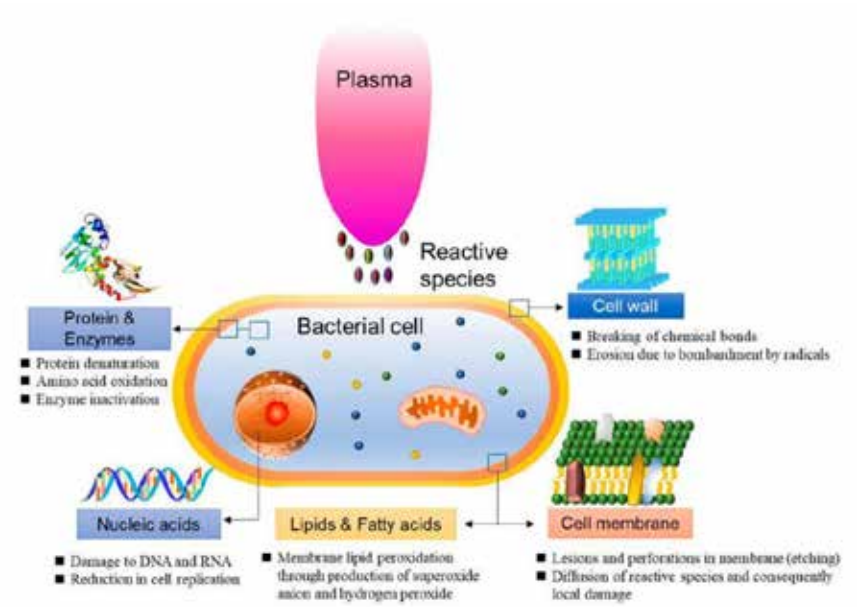

Figure 6: Schematic representation of bacterial reduction induced by CAP[58]

cells (PMN)/granulocytes could be reduced in a dose dependant manner, while PMNs showed enhanced integrin and selectin expression, and an increase of activation markers on their cell surfaces as a result of more CAP treatment.[404]

7.3 What it does: clinical indications

However, when reviewing the fast-growing body of CAP peer-reviewed publications, it becomes clear that the most frequent and prominent clinical observation is the direct antimicrobial efficacy of CAP. In wounds, it is seen to reduce infection and bacterial load significantly, two factors which, according to many, are major contributors to impaired and incomplete wound healing. As can be read in more detail elsewhere in this document, CAP has shown its benefits in both in vitro and in vivo studies, where the reduction of various bacteria and fungi become apparent immediately after treatment.[153,212,405,406] As early as 2010 and 2012, Isbary et al. reported that 2- and 5-minute clinical CAP treatments were able to decrease the microbial load in different types of chronic ulcers, including diabetic ulcers, irrespective of bacterial species.[14,166,172] Due to its strong broad-spectrum antimicrobial ability to reduce microbial loads in wounds effectively, some position CAP as a potential alternative or adjuvant to conventional antibiotics for the treatment of bacterial infections, including those caused by antibiotic-resistant pathogens.[407–409]. Although the formation and presence of biofilms can impair the wound treatment efficacy of CAP significantly, and potential bacterial resistance has been observed, study results still show CAP to be a promising addition for the treatment of biofilm containing wounds.[200,255,406,410–412]

More recently, the efficacy of CAP for the treatment of DFU was investigated in several randomised trails. And although many of these studies were troubled by small population sizes, most did show beneficial CAP treatment effects in terms of bacterial load, wound surface reduction, and time to closure.[178–180,182,186,190,413] It is therefore understandable that the 2022 German guideline, published on the therapeutic use of cold plasma, concludes that the main clinical benefit of CAP is the effective deactivation of microorganisms, including multi-resistant pathogens, followed subsequently by the stimulation of cell proliferation and micro-circulation, resulting in tissue regeneration. Its strong recommendation towards clinicians therefore is to consider the treatment of chronic and infected wounds with CAP.[199]

7.4 Next regulatory step: determining applicable legislation

Medical devices in the EU and European Free Trade Association (EFTA) countries are regulated by the MDR (EU 2017/745) and supervised by the Notified Bodies, augmented sometimes by scientific consultation of the European Medicine Agency.[414,415] Medicinal products, however, are regulated by different legislation which is enforced by European Medicine Agency and the national Competent Authorities direct, i.e. Regulation (EC) 726/2004

and Directive 2001/83/EC, although the latter will be replaced by a new Pharmaceutical Regulation in the near future.[416–420]

Article 1 of Directive 2001/83/EC defines a medicinal product as "(a) any substance or combination of substances presented as having properties for treating or preventing disease in human beings; or (b) any substance or combination of substances which may be used in or administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis".[417]

Looking at Article 2 of the MDR, it defines a medical device as "any instrument, apparatus, appliance, software, implant, reagent, material or other article intended by the manufacturer to be used, alone or in combination, for human beings for one or more of the following specific medical purposes: diagnosis, prevention, monitoring, prediction, prognosis, treatment or alleviation of disease, [...] and which does not achieve its principal intended action by pharmacological, immunological or metabolic means, in or on the human body, but which may be assisted in its function by such means".[414] From this, one could conclude that both definitions are mutually exclusive.

This means that in practice, whether in the USA or in Europe, regulators will always first attempt to determine which legislation applies, determining whether the submitted application should be regarded a medicinal product or medical device. Furthermore, this classification will also define which authority will be responsible for its evaluation, potential approval, and continued review after market entry. In their 2020 comprehensive review, von Woedtke et al. listed the EU available clinical plasma devices as medical devices, CE certification Class IIa according to the European Council Directive 93/42/EEC.[421] This directive, also known as the Medical Device Directive, was, however, superseded in May 2021 by Regulation (EU) 2017/745, the MDR.[414,422] This is relevant, since all medical devices approved in the EU under the former Medical Device Directive 93/42/EC, will need to obtain renewed market approval, as defined in Article 120 of MDR and its later amendment, Regulation (EU) 2023/607.[414,418,423–426] Streamlining this process, submission for approval is now possible at a pan-European level, offering a more efficient route to the previous national level regulatory submission.[427,428]

7.5 Innovation at the edge of regulation

Regulatory frameworks are, due to their pre-defined and reactive nature, challenged continuously in everyday practice by the introduction of new therapies, especially when such therapies transcend pre-existing regulatory axioms and definitions due to their innovative approach.[429,430] Paradoxically, that can mean that innovative therapeutic solutions, critical to address strong unmet medical needs, are halted or delayed significantly on their way to the bedside of patients. In order to anticipate this, the Borderline Manual by the Borderline and Classification Working Group (BCWG) can be consulted.[431–434] The BCWG is chaired by the European Commission, and consists of representatives of Competent Authorities, the national medicine agencies of all member states, and a number of stakeholder associations as observers.

In short, the Borderline Manual is a record of previous cases where the classification of a submitted new product was unclear, and therefore was discussed individually within BCWG. In instances where all guidance is inconclusive, applicants, who are unclear on the correct classification of their product, should consult a national Competent Authority, and provide information on their product's composition and constituents, a scientific explanation of its mode of action, and its intended indication for use. National Competent Authorities subsequently classify borderline products either as medicinal products or as medical devices on a case-by-case basis.[428] With remaining uncertainty or dispute, cases can, and have been, submitted to the European Court of Justice for final deliberation and verdict, thus creating jurisprudence.[435,436]

This process, and all its challenges in real life, can be illustrated for a new imaginary, but highly innovative CAP wound treatment device. As outlined, the first question for such a device to answer would be whether the device should be regarded a medical device or a medicinal product. As described by von Woedtke et al. and CAP device manufacturer data, currently CE-marked CAP devices are approved as either medical devices class IIa or class IIb.[421] The definitions of medical devices class IIa of the former Medical Device Directive, and the MDR now in force, do not differ significantly. According to MDR, "all non-invasive devices which come into contact with injured skin or mucous membrane are classified as [...] class IIa if they are principally intended to manage the micro-environment

of injured skin or mucous membrane".[414] Although the earlier mentioned publications indeed do show that CAP seems to manage the micro-environment successfully, one could reflect on whether its clinical efficacy induces actively chronic wounds to close. If indeed our innovative CAP device does induce wound closure actively, the clinical efficacy of CAP could be more in line with the definition of a class IIb medical device, i.e. "[...] class IIb if they are intended to be used principally for injuries to skin which have breached the dermis or mucous membrane and can only heal by secondary intent".[414] The overarching question here, however, might not be whether the primary clinical wound healing efficacy of CAP is passive or active, but more fundamental, whether CAP "does not achieve its principal intended action by pharmacological, immunological or metabolic means, in or on the human body, but which may be assisted in its function by such means".[417]

Based on the available literature and guidelines, it is evident that the antimicrobial properties of CAP are regarded not as its sole, but certainly as its most prominent, clinical effect. And although all previous reports mention that the exact mechanism of action is unclear and subject to further study, the majority identify ROS as an active, and in some instances, dose-response dependant clinical component.[102,396]

7.6 The Borderline Manual

In cases of regulatory uncertainly, one of the first actions is to compare to previously approved products, decisions and, in some cases, jurisprudence. Following this standard procedure for the imaginary CAP device, one can find that, in the 2019 edition of the Borderline Manual, antimicrobial photodynamic therapy (APDT) disinfection systems were discussed. APDT is intended for the decolonisation of potentially-pathogenic bacteria, including MRSA, from the oral cavity or anterior nasal passages.[431] The photosensitiser solutions used in APDT systems produce ROS, which are responsible for lethally disrupting the microbial cell wall. Since the primary action of the photosensitisers is not physical, their activation of anti-bacterial efficacy is not achieved via physical means, and they are sold typically separate from the energy supplying/ activating (laser) device, they were judged to not be medical devices. The lasers activating these ROS producing photosensitisers, however, were marked as a medical device class IIa.[431] Although the involvement of ROS in this case makes it appear comparable to our imaginary CAP device, the fact that the ROS producing photosensitisers are a separate, independent component, makes comparability, and thus applicability questionable.

The 2022 editions of the Borderline Manual seem to give more guidance. This time, the Medical Device Coordination Group (MDCG) 2022-5 notes carefully that although "not an exhaustive criterion, the presence of a dose-response correlation is indicative of a pharmacological, metabolic or immunological mode of action",[432] while the December edition of the Borderline Manual of that year discusses another ROS based disinfectant.[433] In the latter case, the outcome reads that "the antimicrobial action of ROS, which is considered as the principal intended action, should be considered pharmacological, immunological or metabolic mode of action. The decision of ECJ ruling 6 September 2012, case C-308/11, also supports that such antimicrobial actions on the human body should be considered pharmacological. Consequently, considering the principal mode of action, this product should not be qualified as a medical device".[434,436]

Further complicating matters is the conclusion that, as pointed out by the MDCG, in the discussed cases the ROS (generating) components can be viewed as separate products which can be purchased separately. In the case of CAP, however, the ROS-containing plasma is only available, and can only be generated in conjunction with a plasma-generating device. Due to this, some might suggest that CAP devices might be combination devices as described in Special Rule 14 of MDR: "All devices incorporating, as an integral part, a substance which, if used separately, can be considered to be a medicinal product, as defined in point 2 of Article 1 of Directive 2001/83/EC, [...], and that has an action ancillary to that of the devices, are classified as class III".[414] Such a classification would indeed recognise the disinfecting ROS aspect of CAP plasma, but struggle to show how it could be used as a separate substance.

Another approach can be to consider the mode of action of CAP devices as not of a ;pharmacological, immunological or metabolic; but as of a physical nature. Examples of this can also be found in the Borderline Manual. The May 2022 edition states that gases intended to be used in anaesthesia and inhalation therapy are regarded medicinal products. Should these gases, however, be used with a physical mode of action, e.g. inflation during minimal access surgery,

then these products can be regarded medical devices. Then again, later, confusingly, it can also be read that "the intention of the manufacturer regarding the action of the substance on the device or on the body is irrelevant for the decision on whether the substance would be considered a medicinal product, because intentionality is not mentioned in the MDR legal provisions under discussion".[432]

Finally, in the latest December 2022 edition of the Borderline Manual, there is a more CAP comparable, but not fully compatible, case.[434] Here, the MDCG discusses whether Ar coagulation units should be regarded as medicinal products or medical devices. These units are used in APC, a mono-polar electrosurgical technique, where the Ar plasma functions as the application electrode, making the intervention contactless. In their evaluation and outcome, the EU Competent Authority representatives within the BCWG conclude that Ar coagulation units are active therapeutic devices. More specifically, BCWG notes that such units influence directly the APC, where electrical energy is administered to body tissues by an Ar plasma stream, thus functioning as an application electrode. Based on a risk evaluation considering the common site of application and nature and density of the applied energy, BCWG classified Ar coagulation units as class IIb medical devices according to Rule 9 of MDR.[434]

7.7 The right regulation: checks, balances and choices

In order to become a new, useful tool at the bedside of patients instead of vapourware, new wound healing solutions must obtain their regulatory and market access approval expeditiously. After all, without the proper regulatory approval, a therapy can be life- and world-changing, but will never reach the patients it is supposed to help. With this thought in mind, one can ruminate on whether the current regulatory frameworks are flexible and adaptive enough to facilitate new and innovative therapeutic developments. As the imaginary new innovative cold plasma wound healing device example here has shown, the EU regulatory approach could hold an inherent rigidity which might not facilitate the introduction and availability of new effective wound healing therapies necessarily. Then again, some will argue rightly that regulatory frameworks and authorities first and foremost should safeguard a stable, albeit less flexible, base where new products just need to fit in and comply with the already defined and applicable standards and policies. Either way, the main objective must remain to provide patients with optimal care in treating their morbidity, and thus enabling their physicians to do so safely with the most effective tools possible. With this prime directive in mind, it seems self-explanatory and imperative to adjust a regulatory framework continuously, albeit vigilantly, to welcome never-before-encountered therapeutic approaches. Because, as the groundbreaking initiative by FDA has underlined again, ever increasing incidence, prevalence, and healthcare budget impact numbers do call urgently for new, inventive, multidisciplinary and effective solutions for chronic, non-healing wounds.[384] It would therefore be exciting to see European regulatory authorities follow the lead of their American counterparts, and extend their continuous effort and commitment to ensure a "sound and flexible regulatory system", as defined by the European Commission in their Pharmaceutical Strategy for Europe, to include the globally recognised unmet need of non-healing wounds.[415]

8. CAP in every day clinical wound practice

8.1 The potential of CAP as therapy for wound healing

Despite the fact that plasmas have been known for more than a century (paragraph 7.2), developments in plasma medicine only picked up speed in the 1990s with the improved availability of CAP technology for antibacterial treatment of medical devices, surfaces and materials (Chapter 2). The application of CAP to non-healing wounds and infections is one of earliest realised medical applications of CAP, with publications on clinical studies from 2010 onwards.[166] These studies describe a broad range of wounds and protocols, with studies becoming more sophisticated over time (Chapters 3–5).

Studies of new technologies in wound healing are notoriously difficult. Conducting clinical trials at scale within a representative population, with suitable randomisation, blinding of participants and patients to the intervention, and inclusion of a control arm, is a difficult process even for the most established technologies. This is further complicated by selection of suitable endpoints. To aid with this process, ample standards and guidelines provide guidance for trials in wound healing.[1,437–441]

For CAP, this has meant that the approach into wound healing has been gradual, with many of the first studies resembling clinical case studies, later followed by more standardised, albeit frequently small scale, open label investigations. In 2023, Li et al. reported in the *BMJ* that they opened the undertaking of systematic review and meta-analysis of studies to date in CAP in wound healing.[413] They note that, although studies of CAP in DFU report that CAP could improve wound healing speed compared to conventional therapy, to date, studies "are small and not sufficiently representative". Nevertheless, the potential of CAP for treating hard-to-heal wounds has not gone unnoticed by the clinical community.

8.2 Working with CAP devices

Despite many clinical studies conducted and planned (Chapter 4–6), various issues remain to be addressed with regard to CAP since they might complicate and hamper its implementation in every day clinical practice. These open questions do not only concern academic studies regarding, for example, the exact mode of action, but also more practical queries which might be considered by wound healing HCPs.

In the previous chapters, an overview of the various types, modes of operation and specific characteristics and limitations of CAP devices was given (Chapters 2 and 5). A consideration for everyday therapeutic operation of medical devices is, however, also their practicability. Devices generating CAP can vary in size, geometry and technical setup, not seldom depending on the intended use.[442] This might not be an issue for the stationary treatment of inpatients or ambulant patients able to visit a practice, but could present a bulky confutation for many regions where post-hospitalisation wound care and the treatment of non-healing wounds is performed mainly as in-home care. However, with device design progressing continuously, smaller more mobile CAP devices suitable for home care have become available, enabling care givers more flexibility in their wound healing treatment (Figure 11).

Another consideration can be that efficacy can be impacted considerably when a device is less flexible and/or suitable for the large variety of wounds patients present to the wound healing HCPs at their practice. For example, a flat CAP patch might not be well suited for wound cavities or very small wounds, while a hand-held PJ will prove impractical for wounds of larger sizes (Figure 7). Furthermore, case studies and RCTs tend to focus on results of CAP treatment of chronic wounds. Unfortunately, the in-practice ever-present variations and limitations with regard to local wound conditions are commonly under-reported. With this in mind, it is essential that HCPs weigh in the physiological and pathological variability that every patient will present inevitably, which here also means that CAP cannot be a 'one size fits all' wound therapy. Practically, CAP should be regarded as a valuable addition for the treatment of chronic wounds, which, like every medical intervention, will need tailoring, evaluation and potential adaptation by experienced wound care HCPs for optimal efficacy and result.

Another of such clinical considerations is the penetration depth of CAP, an ongoing task in plasma medicine research, especially since the distribution of bacteria in chronic non-healing wounds has been seen to vary significantly.[443] When reviewing, a distinction must be made between real penetration of reactive species into deeper liquid or tissue layers, respectively, and in-depth biological effectiveness (Table 9). Diffusion of plasma-originated reactive species depends strongly on the reactivity of the species itself, as well as the characteristics of the (biological) environment, and the content of potential reaction partners. Available in vitro research does, however, suggest that the physical effects of plasma can be seen to depths of several hundred micrometres within tissues. Plasma-derived RONS are likely delivered into tissues for millimetres, since speciation reveals that RONS delivered by plasma into tissues and tissue fluid are predominately stable secondary RONS (e.g. H_2O_2, NO_2–, and NO_3–).[18,26] Furthermore, plasma generation of RONS within a hydrated target is influenced by the target matrix, which can enhance or reduce the RONS concentrations and act as a reservoir of RONS. This suggests concentrations exceeding hundreds of micromoles, even at depths of several millimetres within tissue.[395] This behaviour might be useful, especially since Melone et al. estimated that at least 78% of all chronic wounds are covered with biofilm or slough.[444] Therefore, pre-procedural debridement as part of a CAP wound treatment plan is advised strongly since it will increase CAP efficacy by better targeting and killing of any remaining (biofilm) bacteria in the wound bed and wound margin.[410,412,445]

8.3 CAP and modalities of treatment

With a significant number of studies conducted in chronic wounds, the optimal treatment duration and frequency have proven difficult to establish. In practice, CAP device experts should instruct HCPs on the appropriate operation, since results can and will vary per device, or indeed per subsequent wound treatment (for some real-life

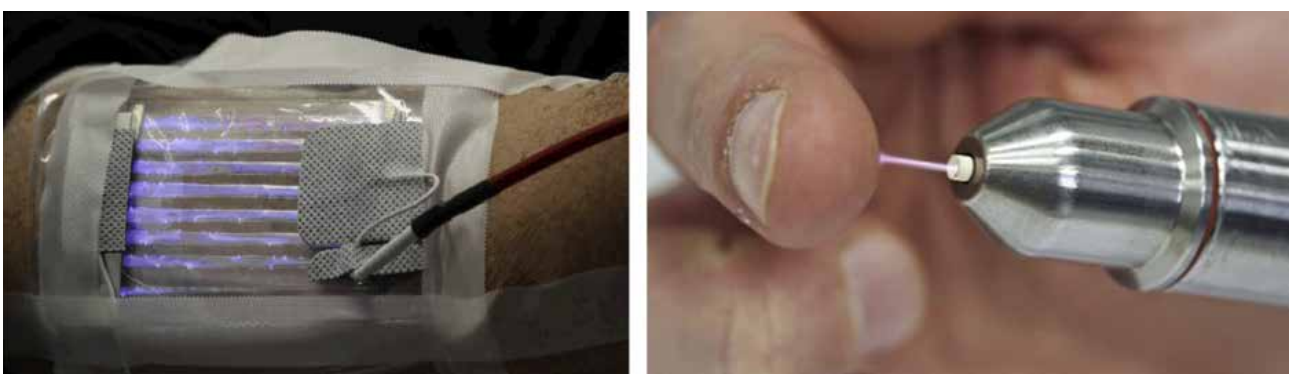

Figure 7: The efficacy of different device types.

Left) investigative flexible SDBD prototype (8.5x13cm^2) applicable for treatment of larger curved surfaces, placed here on the proximal anterior forearm (INP Greifswald, Germany);[13] Right) an Ar PJ.[360]

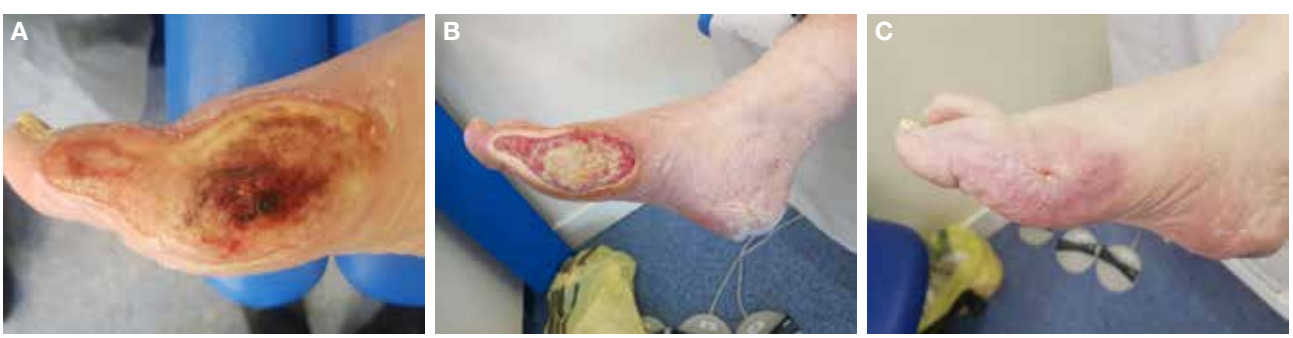

Figure 8: Wound healing example: 80-year-old male.

Male, 80 years old, with persistent, multidrug-resistant infected DFU for 19 months. Treated with microwave Ar CAP (Adtec Healthcare Limited® SteriPlas™), in combination with standard care (no antibiotics) for 16 weeks. Treatment frequency: once a week for 3 minutes. Clinical situation at: A) therapy start; B) after debridement and first treatment; and C) 16 weeks of treatment. Kettering General Hospital NHS Foundation Trust, United Kingdom.

examples, see Figures 8–11). Accordingly, no evidence-based standards on plasma application and therapy frequency exist to date.[199] No doubt due to the broad range of different plasma technologies, devices and clinical study related variables, CAP treatment durations from 30 seconds per cm^2 up to 7 minutes have been reported (Table 6). Furthermore, CAP treatment schedules varied from just once a week, multiple times per week, to even repetitive use up to five times a day over a period of several weeks. Although it is difficult to generalise due to the earlier mentioned variability, there seem to be indications that CAP wound treatment once or twice a week at the most might be sufficient,[446] while for sole antimicrobial purposes, a more frequent treatment, once daily for 1 week, is suggested.[166] This therapeutic approach is confirmed further by the observation of Moelleken and colleagues that results of a once weekly treatment were not inferior to those for CAP treatment three times a week. For such a regimen, investigators also found that treatment once a week was organisationally easier and more economical to implement in clinical routine.[446]

When considering optimal dosing in the everyday clinical practice for wound treatment, it is worth mentioning that clinical observations report that prolonged exposure of the skin/wound can potentially cause adverse effects such as dehydration, hypoxia and skin damage.[137,143,149,447] Another basic therapeutic consideration might be how to minimise heat transfer to the patient, thus preventing potential thermal tissue damage and increased erythema.[212] The

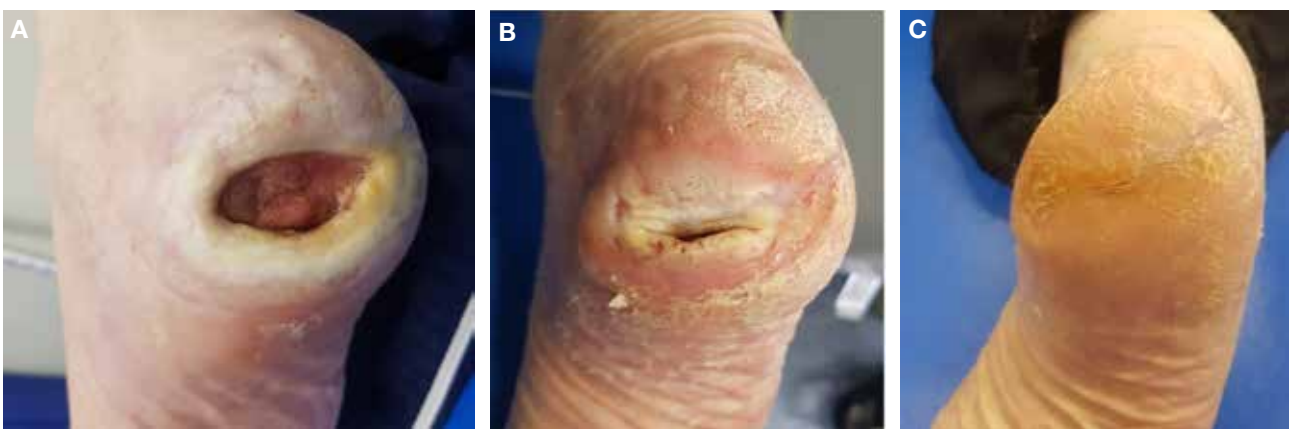

Figure 9: Wound healing example: 80-year-old male.

Male, 80 years with persistent, multidrug-resistant infected DFU for 19 months. Treated with microwave Ar CAP (Adtec Healthcare Limited® SteriPlas™), in combination with standard care (no antibiotics) for 56 weeks. Treatment frequency: once a week for 3 minutes. Clinical situation at: A) therapy start; B) after 24 weeks; and C) 56 weeks of treatment. Kettering General Hospital NHS Foundation Trust, United Kingdom.

Figure 10: Wound healing example: 68-year-old female.

Female, 68 years with persistent DFU for more than a year. Treated with VDBD CAP (ColdPlasmaTech® CPT®patch), in combination with conventional treatment for 8 weeks. Treatment frequency: twice a week for 2 minutes. Clinical situation at: A) therapy start; and B) after 4 weeks of treatment. Martin Luther Hospital, Berlin, Germany.

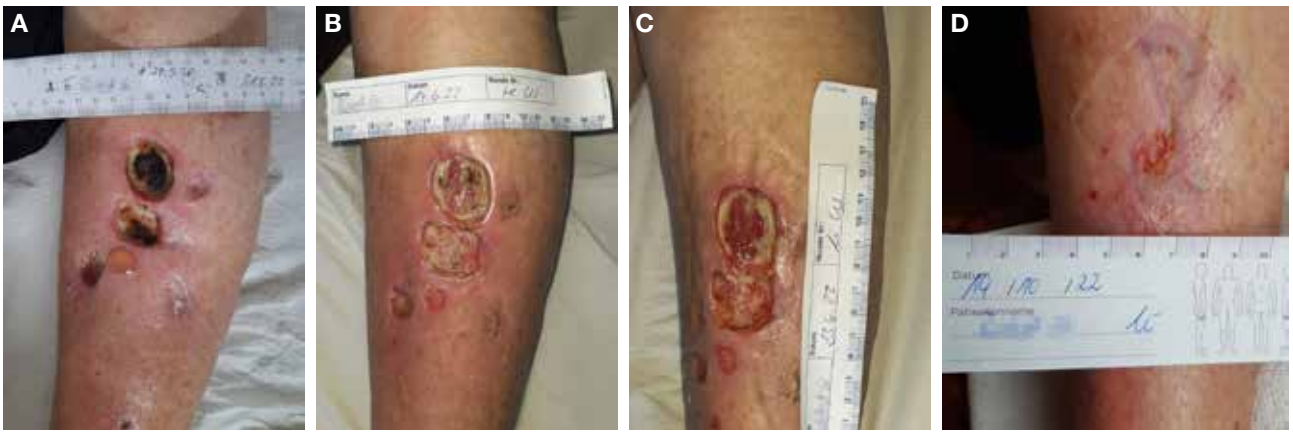

Figure 11: Wound healing example: 84-year-old female.

Female, 84 years with persistent infected VLU for more than a year. Treated with VDBD CAP (ColdPlasmaTech® CPT®patch), in combination with conventional treatment. Treatment regimen: twice a week for 2 minutes. Clinical situation at: A) therapy start; B) after debridement at 2 weeks; C) after debridement at 4 weeks; and D) after 20 weeks of treatment. MeckCura Plegedienst GmbH ambulant/ homecare, Rostock, Germany.

issue of potential plasma induced heat damage to tissues will be addressed in a new, upcoming standard, a revision of the earlier German standard DIN SPEC 91315:2014-06 – General Requirements For Plasma Sources In Medicine (publication planned for 2024). Contrary to the earlier 2014 standard[448] which focused on plasma temperatures exclusively, this revision will focus on the more relevant aspects of tissue temperature as a result of energy transfer from the plasma to the tissue, using ex vivo models in combination with optical methods for temperature quantification. Manufacturers of medical plasma devices have been aware and diligent of these treatment risks during the product development of their devices, subsequently integrating technical measures in the product design, preventing patients from excessive energy input, thus keeping temperature increases under the maximum temperature of 39°C as specified by the earlier German DIN standard.[448] For some of the medical plasma devices available on the market currently, this means that their (flat) sources produce sufficiently low tissue temperatures, mainly by specific electric signal characteristics, while alternative jet-like configurations apply physical spacers to prevent direct interaction of 'hot' parts of the plasma with the patient's tissue. Nevertheless, it remains important to optimise the plasma dose, and find the delicate balance between both maximised CAP efficacy and minimised AEs in the wound and adjacent healthy tissues.[301] Here, more clinical studies can and will help to gain further insight into the optimal treatment modalities for specific patient characteristics.

8.4 CAP reaching the patient with chronic wounds

Even though the clinical wherewithal of CAP for the treatment of chronic and hard-to-heal wounds is most promising, it can only unfold its full beneficial potential when patients have optimal access to said treatment. Where nowadays more and more younger patients (<65 years) present themselves with chronic and hard-to-heal wounds due to the rise of lifestyle disease, the typical chronic wound patient is (still) elderly and multi-morbid. This causes a sluggishness in visiting outpatient clinics and hospitals several times a week, the location where CAP treatments are mainly performed currently. Multiple weekly applications, such as those for antimicrobial wound therapies, must be considered difficult in this context.[446] Therefore, sufficient evidence, mapping the efficacy of CAP, and investigating especially the minimum effective dose (e.g. once-weekly application to the wound) is urgently needed. Furthermore, the availability of affordable, small, portable devices, such as for example an air-fed CAP device already used to treat residual tumour cells in surgical cavities,[216] would increase the therapeutic impact of CAP significantly. As it stands, CAP application by appropriately trained HCPs will most likely be of benefit to wound patients who are expected to gain the largest increase in QoL by CAP treatment. Having said this, however, even with CAP as an addition to the wound healing toolbox to control the bacterial burden of a wound, it should not invite HCPs to not diagnose and treat the underlying (causal) morbidity, as seen frequently in Europe today.[449]

9. Appendix I: The physics of CAP

9.1 Introduction

This chapter aims to help develop a basic understanding of the physics relevant to CAP. For this purpose, mathematical equations are avoided deliberately, and the focus is instead on phenomenological descriptions.

CAP is a special variant of physical plasma. In physics, plasma refers to a gaseous state of matter in which the integrity of atoms and molecules is at least partially violated. Being a natural phenomenon, the plasma state is well known to (astro)physicists as 99.9% of the radiating matter in universe. Materials scientists and engineers are well acquainted with physical plasma, having engineered it for a huge variety of (industrial) applications for decades. These applications encompass, for example, surface functionalisation of polymers,[450] generation of radiation,[451] production of semiconductors,[452] thin film coatings,[453] material synthesis,[454] and decontamination purposes of surfaces, liquids and gas streams.[35,455,456]

However, despite vast scientific and research progress, physical plasma's technological contributions to our modern society goes largely unnoticed by the general public and outside of most school curricula. In nature, plasma is not only more visible, but even the fundament of visibility as the main source of natural light in our solar system attributable to the enormous plasma ball in its centre.

In healthcare, the term plasma might be particularly confusing due to established definitions in relation to the circulatory system. In medicine, it usually gives name to the yellowish liquid component of blood that contains water, salts and proteins but no blood cells, white cells or platelets. Furthermore, in cell biology, the cytoplasm refers to all materials enclosed by the cell membrane, excluding the nucleus in the case of eukaryotic cells.

9.2 Physics basics

To understand the nature of physical plasma, it is helpful to briefly refresh three basic topics that most readers will be familiar with from physics or chemistry classes.

9.2.1 Atoms

An atom is the basic particle of chemical elements and consists of a nucleus surrounded by a cloud of electrons. The nucleus holds at least one or more positive charges (protons), while negative charges carried by electrons can be found in the cloud surrounding the nucleus (Figure 12a). When the amount of positive and negative charges is balanced, the net charge of that atom equals zero, and it is referred to as a neutral particle.

When a certain amount of energy, specific for each element, is applied to an atom, one of the electrons may overcome the forces that have bound it to the nucleus, and thus the outcome of this event leaves behind a positively charged ion and a free electron (Figure 12b). This process is referred to as ionisation. In an ensemble of many particles, the released electron, as well as the ion, can be subject to energy sources and interact with other particles.

At moderate energy supply, an electron might not be released, but rather excited to a higher energy state of that element as indicated by the dashed circles in Figure 13. When falling back spontaneously to ground state (relaxation), the energy difference between ground state (Figure 13a) and excited state (Figure 13b) is released in the form of electromagnetic radiation (photon) according to Figure 13c. At a certain wavelength range, the human eye can detect photons and identify this radiation as visible light. More energetic photons are referred to as UV radiation. As an alternative to emitting a photon, the excited particle can also store the absorbed energy for a certain period of time (metastable) and transfer it to other particles via collisions.

9.2.2 Molecules

Atoms can attach to other atoms by chemical bonds, thereby forming molecules. Most relevant are covalent bonds, in which an electron of one atom together with an electron from another atom form an electron pair. Naturally, atoms in the molecular bond can still be subject to excitation processes, eventually leading to ionisation or photon emission, just like non-bounded atoms. In addition, molecules may give up their multi-atom configuration and

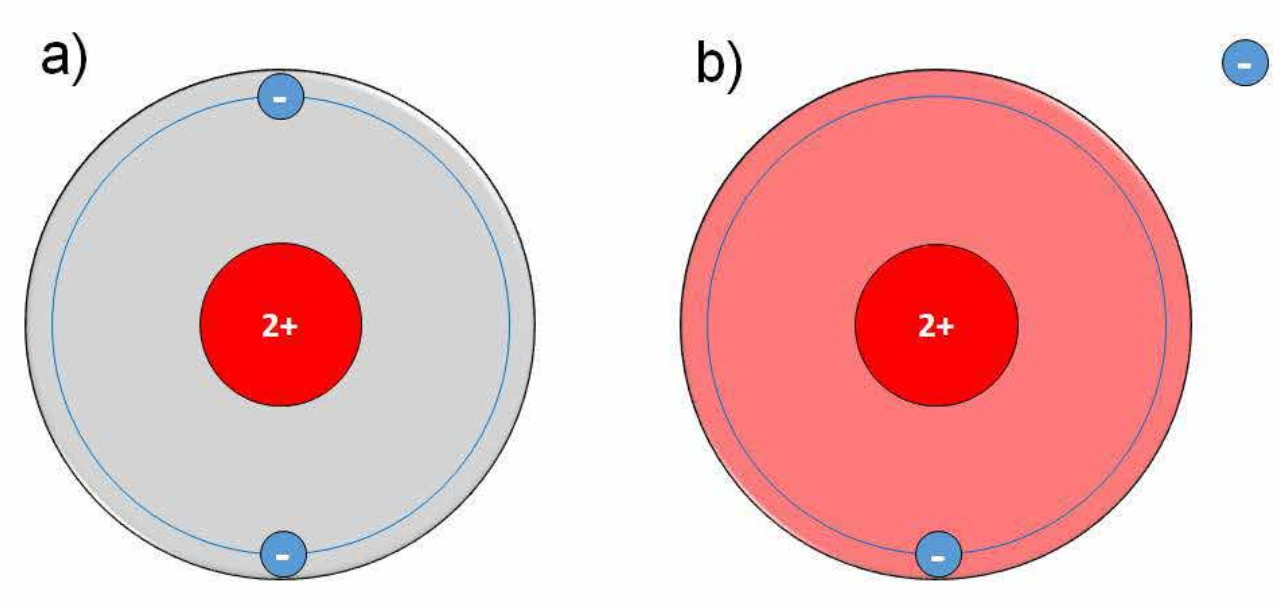

Figure 12: Shell model of the element He.

a) shows He in the neutral ground state configuration (grey); b) shows the first ionisation configuration forming a positively charged ion (red) and a negatively charged free electron (blue), with the latter being no longer bound to that atom nucleus.

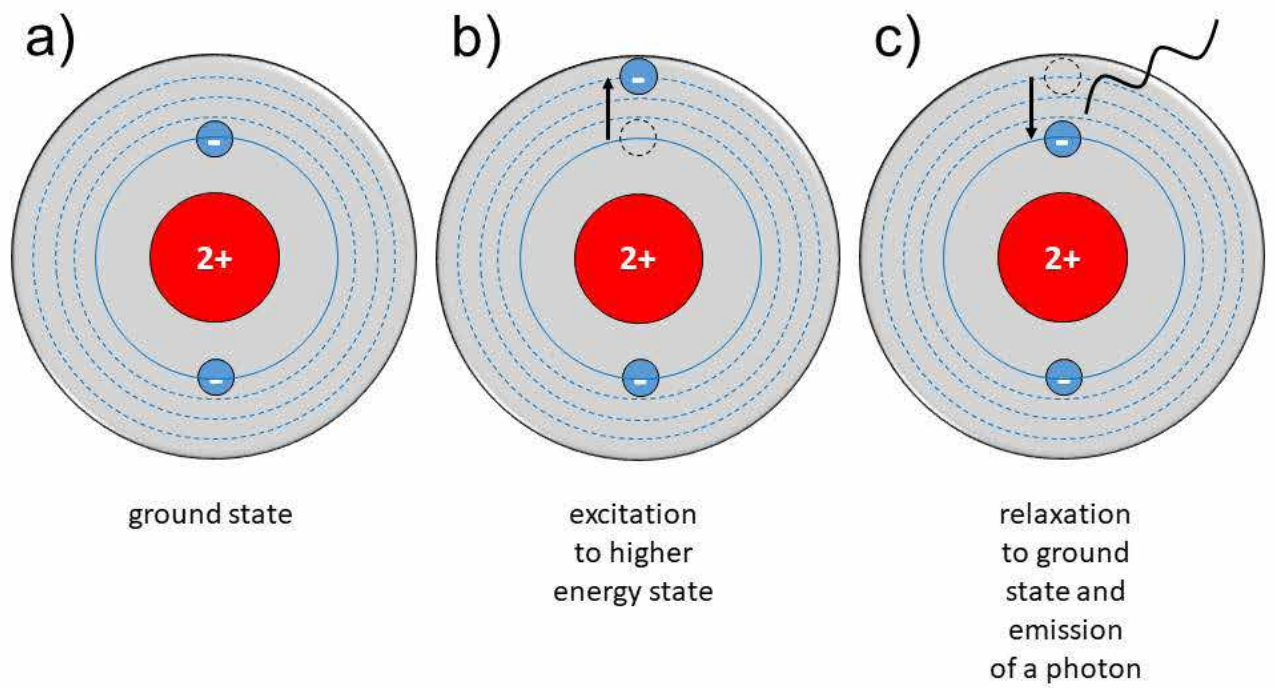

Figure 13: Shell model of the element He with moderate energy supply.

a) shows He in the ground state; whereby b) an electron can be excited to a higher energy level (dashed lines); and c) can relax back to the ground state. The energy above ground state is released in the form of electromagnetic radiation (photon).

decay into their atomic components (dissociation) as soon as sufficient energy is supplied to release the bond. The oxygen molecule O_2 in Figure 14a is the common and stable configuration of two oxygen atoms at atmospheric conditions. As illustrated in Figure 14b, it can be dissociated to two oxygen atoms (O). However, the outer shell of each atom is now missing two electrons, which renders the atomic configuration of oxygen highly reactive, and offers the starting point for gas phase reactions involving other atoms and molecules.

9.2.3 Thermodynamics and particle collisions

Atoms and molecules as building blocks of matter are in constant disordered motion, with a mean velocity of the particles being determined by, and proportional to, temperature and energy. One temperature that applies uniformly throughout an ensemble of gas particles exists only when the system is in a state of thermal equilibrium. Here, the particle velocities are subject to a defined statistical distribution – some particles have higher velocities than the average velocity, while others move at less speed. In air at atmospheric pressure and a temperature of 20°C, about 10^{25} gas particles per cubic metre move at a mean velocity of more than 1600 kilometres per hour and collide with 7 billion other gas particles per second. At constant temperature, each particle of the ensemble of particles transfers energy and momentum continuously with other particles by collisions, but the overall amount of (heat) energy of the system is constant. As soon as energy is added to this ensemble of particles, the energy is

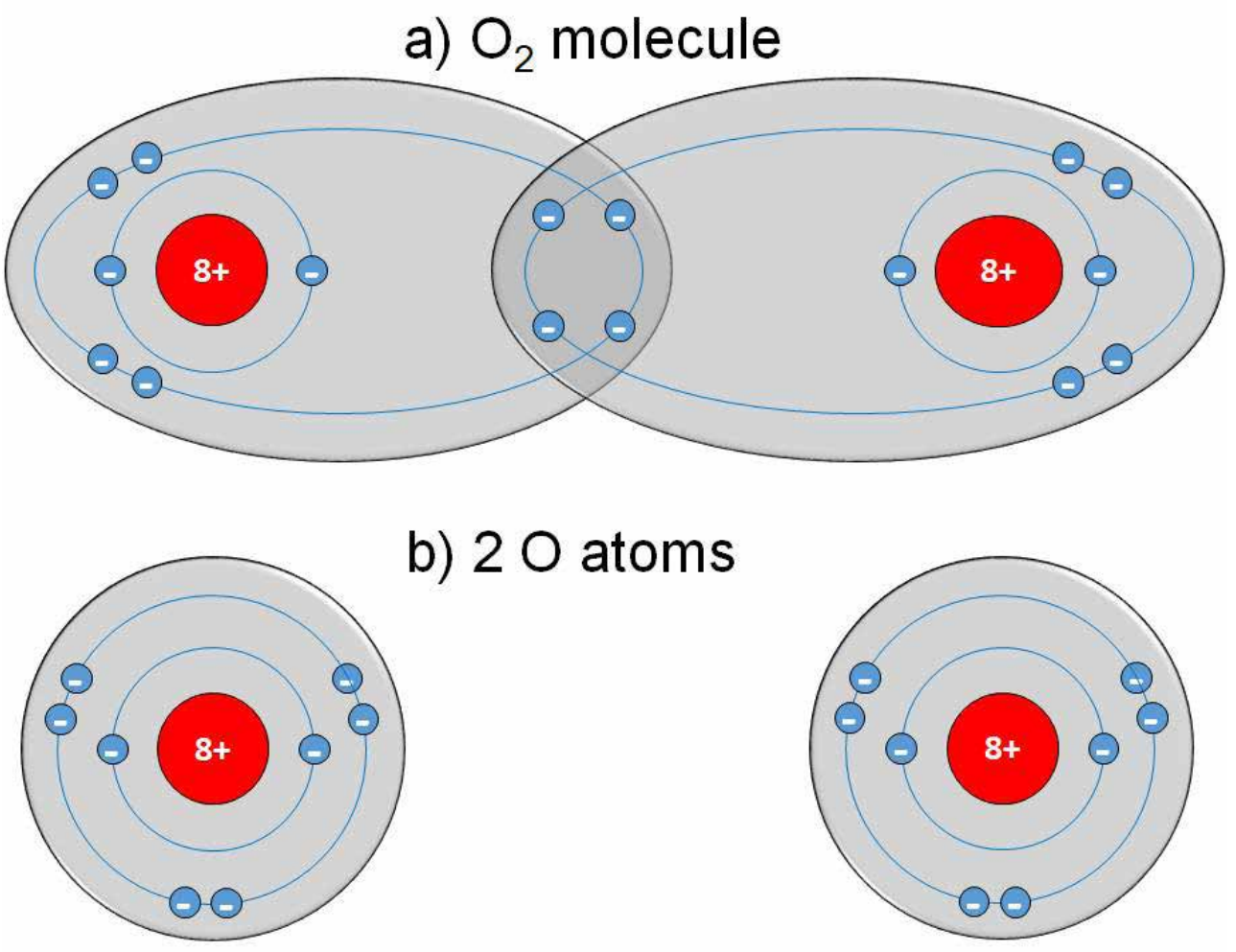

Figure 14: Electron configuration of an oxygen molecule.

(a) shows an oxygen molecule in which each atom shares two of their outer electrons in an electron pair; (b) shows two separate oxygen atoms not interacting with each other.

distributed homogeneously between all particles by (elastic) collisions until the system adapts to thermal equilibrium at a higher temperature than before, which equals a higher mean velocity of the particles.

In our everyday life, we often experience systems in thermal equilibrium, e.g. the evenly warm water of a bathtub, the cold winter air, the hot stove top. But we also know systems that are not in thermal equilibrium, or at least not in thermal equilibrium for a certain amount of time. Deviations from thermal equilibrium often occur when a system is composed of subsystems. As long as the subsystems can exchange energy, the system strives towards thermal equilibrium by itself with time constants depending on temperature difference and efficiency of (heat) energy transfer. An example of such a mixed system on macroscopic scale is a glass of hot tap water filled with ice cubes. Due to efficient energy transfer, the heat of the hot water first melts the ice cubes and then heats the cold water until the liquid in the glass equilibrates at a uniform temperature. This process (thermalisation) does not occur instantaneously but requires a certain amount of time, e.g. minutes for the earlier ice cube example (Figure 15a).

However, if the energy exchange between subsystems is not efficient, it can take much longer to equilibrate. An example of a system where (heat) energy transfer is very inefficient is an insulated mug. Due to its double-walled construction, the heat energy exchange between the inside and outside of the mug is severely limited, so that a cool liquid inside equilibrates with the inside wall rather quickly, but only equilibrates with the temperature surrounding the mug within hours (Figure 15b).

When a system only exists for a certain amount of time, and time constants for thermalisation are significantly above this lifetime, a state can establish with subsystems continuously existing at different temperatures (Figure 15b). An important key for understanding the nature of CAP is that it is a gaseous system, composed of subsystems at the microscopic scale, existing at highly different temperatures.

9.2.4 Physical plasma as the fourth state of matter

Matter can exist in different states, with solid, liquid and gaseous being the classical states (Figure 16). On a microscopic scale, solids are characterised by fixed positions of each atom relative to other atoms because of attractive forces between them. The movement of each atom is restricted to oscillations around the fixed central position, with the intensity of oscillations being proportional to temperature. At a temperature specific for each element and material composition, the forces induced by the oscillations overcome the attractive forces of the atoms, thus provoking the phase shift to a liquid state. In liquid

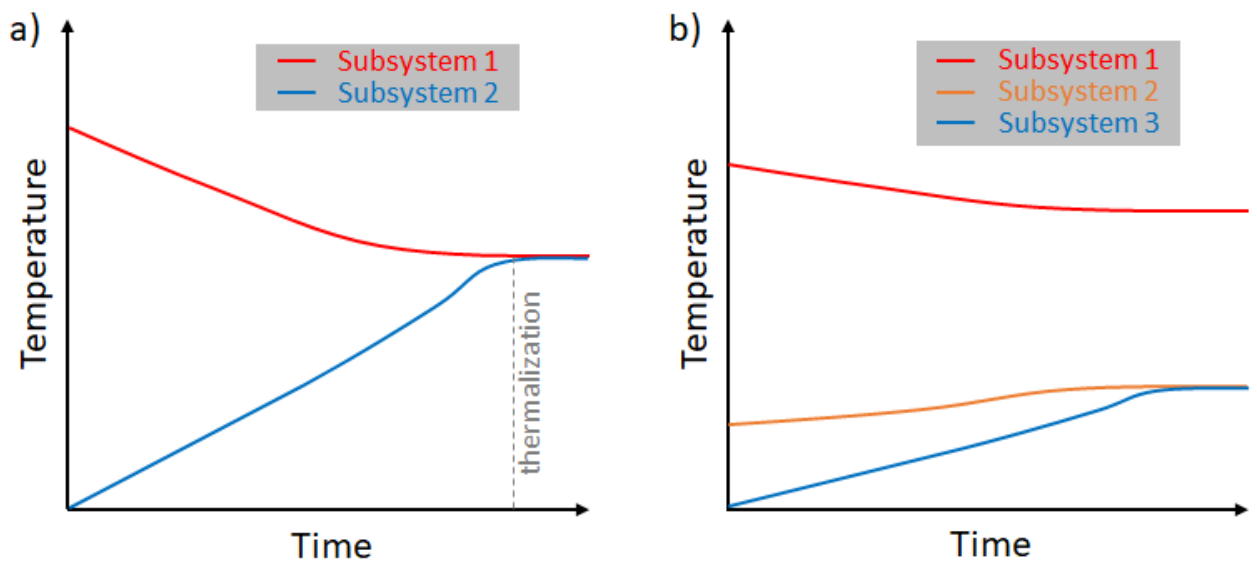

Figure 15: Graphs showing the process of thermalisation.
a) shows the thermodynamic system of two subsystems that equilibrate to the same temperature within a certain time;
b) shows a system consisting of three subsystems in which only two subsystems reach thermodynamic equilibrium.

state, particles can almost move freely and collide with each other, attractive forces between particles being significantly lower compared to solids. With a further increase of temperature, a liquid becomes gaseous. Accompanied by this phase shift is a significant loss in density – the number of particles per volume unit. As a consequence, gas particles can move freely and experience less collisions compared to liquids.

When energy is delivered to an ice cube in the form of heat, its molecular components lose their crystalline long-range order and the solid becomes liquid water. The molecular components of both, ice and water, are still identical, i.e. a molecule formed from two hydrogen atoms bound to one oxygen atom (H_2O). When the temperature is increased further, liquid water evaporates to become gaseous water vapour. Hereby, 1 litre of liquid water can produce almost 1700 litres of water vapour, thus illustrating the massive decrease of particles per volume (density). Chemically, however, the gaseous components are still H_2O. They feature a higher mobility and move at higher speeds, yet the number of electrons in the atomic shells is still identical to the number of protons in the nuclei, thus rendering the solid, liquid and gaseous H_2O molecules electrically neutral.

As soon as the excitation of gaseous neutral particles exceeds a certain energy level, processes such as ionisation, dissociation and photon emission occur. The gas becomes plasma, i.e. a mix of free electrons, gaseous ions, gaseous neutral particles and photons. In terms of thermodynamic properties, all particle classes can be considered a separate subsystem. The temperatures of the electrons, ions and neutrals can be very close to each other (equilibrium) or differ significantly (non-equilibrium). In fact, the temperature depends on how efficient each subsystem is in gaining energy from an energy source after balancing the losses by distributing this energy-uptake throughout the (gaseous) system.

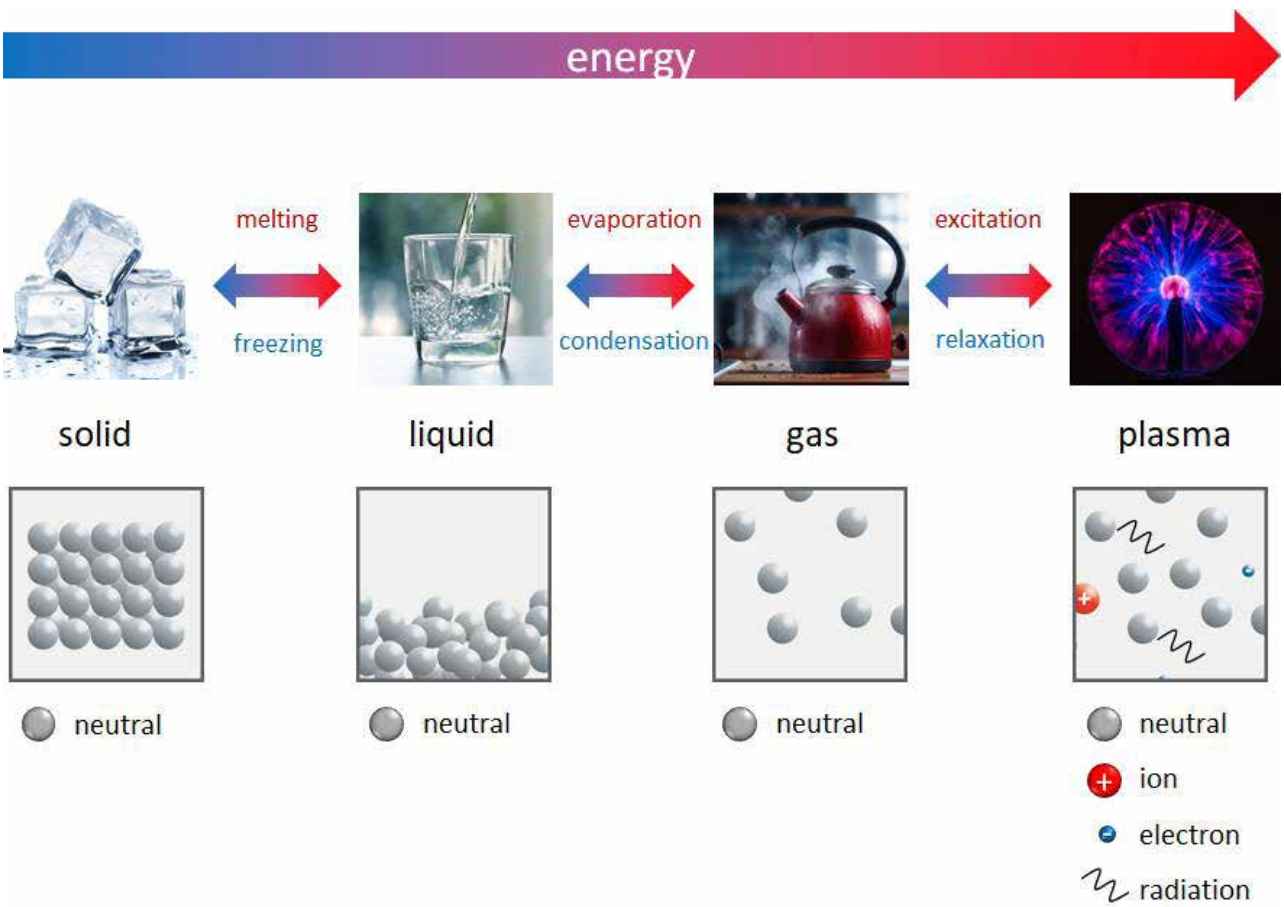

Figure 16: The three classical states of matter with their arrangement and density of neutral particles in comparison to the plasma state as a mix of neutral and charged particles as well as electromagnetic radiation (photons).

The degree of ionisation, which corresponds to the ratio of charged particles to neutral particles in a plasma state, can vary strongly in the range from only a few particles per million up to a fully ionised system. As a direct consequence of the presence of free charge carriers, plasmas carry electric currents and can be manipulated by electric and magnetic fields. They can also exist at a wide range of pressures and temperatures. After all, plasmas glow, and the spectral characteristics of the luminous appearance depend on the base gas, pressure and temperature.

9.3 CAP

CAP is a special variant of a physical plasma characterised by the following attributes:

- it operates close to atmospheric pressure;[457]
- the energy required to generate and maintain the plasma is supplied as electrical energy by so-called gas discharges;[303]
- particle collision is the dominant mechanism of energy transfer in the plasma;[458]
- the vast majority of the particles present are neutral particles, while ionised particles (electrons and ions) exist only at approx. one in a million;[459]
- the mean temperature of the electrons, corresponding to their energy, is orders of magnitudes higher than the mean temperature of ions and neutral particles;[460]
- heat transfer to the treated area is reduced to the technically possible minimum.[314]

CAP is a mixture of components existing simultaneously in a confined space as a result of supplying energy to a gas. Without energy supply, the plasma extinguishes, allowing CAP to be turned on and off virtually at the push of a button. As illustrated in Figure 17, CAP encompasses neutral gas particles that represent the main portion of particles. In addition, there are charged particles, such as electrons and ions, deriving from ionisation processes. Excitation and relaxation processes produce electromagnetic radiation (photons), mainly in the UV and visible spectral band. With molecular gases included, dissociation processes lead to the formation of reactive gas species. Since ambient air is always involved in medical applications of CAP, many of these reactive species belong to the group of RONS.[461] Finally, the energy for CAP development derives from strong electromagnetic fields. Therefore, these fields can also be considered a component of CAP. Together with the

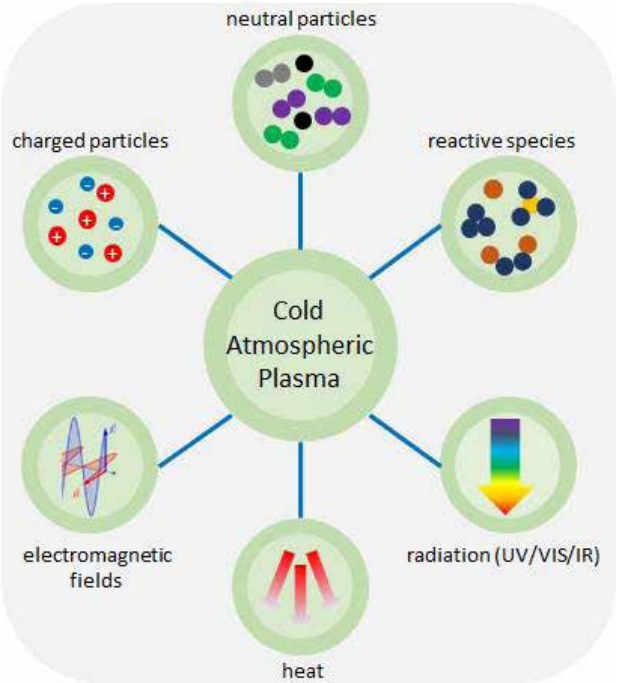

Figure 17: CAP as a mix of different components.

electrical current flow and heat conduction induced by the plasma, electromagnetic fields can also have an impact on medical treatments by increasing tissue temperature.

9.4 Gas discharges

Common for the generation of physical plasmas are electrical gas discharges. A variety of technical concepts exists to generate CAP at atmospheric pressure.[462] However, the basic physical mechanisms in the gas phase are comparable in all concepts. A simple configuration to illustrate the concept of a gas discharge is an arrangement of two parallel plates (electrodes), made from electrically conducting materials, arranged at a distance of millimetres to centimetres as depicted in Figure 18.

Each electrode is connected to one pole of a voltage source, indicated typically by positive and negative polarity. The voltage induces an electric field in the gas volume that accelerates seed charge carriers by Coulomb forces. These seed charge carriers are sufficiently available through natural processes in habitable layers of the earth atmosphere. Specifically, positively charged ions are accelerated towards the negative electrode, while electrons gain speed on their way to the positive electrode. The stronger the electric field, the higher the velocity gain of the

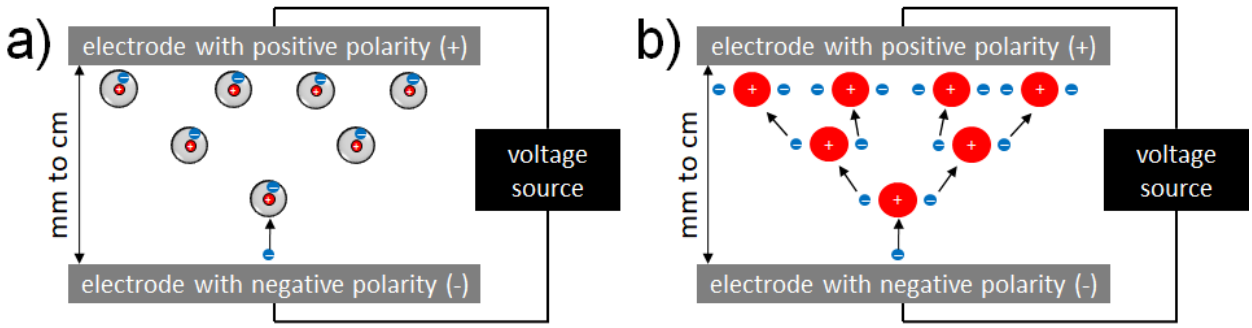

Figure 18: An example of electron impact ionisation of neutral particles.

Electrode arrangement with a) seed electron (bottom) inducing b) an electron avalanche in the gas volume between two oppositely charged electrodes.

charge carriers per distance unit. Given balanced charges, neutral particles are not accelerated and cannot absorb energy directly from the electric fields. However, collisions with charge carriers that have previously absorbed energy in the electric field is a mechanism by which part of the field energy can also be transferred to neutral particles.

At the very beginning of each gas discharge, a naturally existing seed charge carrier (typically an electron) gains energy from the electric field (Figure 18a). In collisions with neutral particles, the seed electron loses part of this energy. However, immediately after the collision, the seed electron again receives energy from the electric field, so that the cycle of energy gain and loss continues. Electrons loose only a small amount of energy in collisions that heat up the much heavier neutral particles (elastic collision). However, given enough energy, they are very efficient in transferring energy to the electrons of the neutral particles (inelastic collisions). At an energy transfer specific for each element, the seed electron is finally able to release another electron from a neutral particle in a mechanism, referred to as electron impact ionisation. The released new electron, just like the seed electron, is also accelerated in the electric field, and on its turn can ionise further neutral particles on its way to the positive electrode. In a chain reaction called electron avalanche, this process repeats until finally an electrically conductive (ionised) gaseous volume forms between the two electrodes – the physical plasma (Figure 18b).

For clarity, the illustration in Figure 18 is limited to one of the most important mechanisms in a gas discharge, i.e. electron impact ionisation of neutral particles. There are further processes, however, which are relevant for the formation of CAP. The impact of electrons with neutral particles can also result in excitation of the particles (metastable) or, eventually, the release of a photon. In the case of molecular gases, electron impact may also lead to rotational and vibrational excitation, as well as dissociation into atoms, which marks the starting point for chemical gas phase reactions leading to the formation of reactive species.[463] All the aforementioned mechanisms induced by electron impact happen simultaneously in a plasma, each of them with different probability, essentially depending on the electron energy and the gas composition. Electron energy of the plasma can be controlled by parameters such as electrode configuration, signal characteristics of the power supply and gas composition. While these parameters are systematically researched during development, market-ready medical devices dispose a fixed electrode configuration that is supplied with constant electrical signal characteristics and operate in a defined gas composition comprising of either a combination of working gases with ambient air or pure ambient air.

As mentioned before, all charge carriers gain energy in electric fields. This also includes ions, whose mass is almost identical to the mass of neutral gas particles. Therefore, (elastic) collisions of ions with neutral particles can increase very efficiently the temperature of neutral particles, while simultaneously decreasing the ion temperature. At the very beginning of each gas discharge, the neutral particles are at ambient temperature and continuously develop toward thermal equilibrium with the ions. In weakly ionised plasmas such as CAP, the number of ions is orders of magnitudes lower compared to neutral particles. Consequently, the temperature where both subsystems reach thermal equilibrium, the gas temperature, is significantly below the initial ion temperature and above ambient temperature.

10. Appendix II: Available and emerging solutions for delivery of CAP therapies in wound healing

Plasma medicine has emerged as a promising field for various medical applications, particularly in wound healing. To pave the way for these applications, innovative plasma technologies have been developed, certified and declared conformant for medical use. Thereby, different technology pathways were applied to make the devices safe for medical use. While first plasma devices are certified under the MDR, ongoing innovations continue to expand the potential applications of CAP therapy.

10.1 Basic requirements for CAP sources in medical technology

Plasma sources are comprised of essential components before a device is operational and available for therapy in plasma medicine. A base unit includes various parts, but mainly provides power for a pre-defined duration of treatment and thus defines the manufacturers studied and approved 'dose control' for efficacy and safety reasons. In the case of systems requiring a working gas supply, a flow control system is similarly implemented into the base unit. A user interface either as part of the base unit or the hand-held device manages the device operation for on/off switching and error signalling. Although in theory many device operation parameters could be made variable as it is typical in laboratory settings, when facing utilisation in every day clinical practice, however, complexity is mostly reduced to an on/off functionality.

In different cases, some manufacturers offer a device family with a variety of individual ergonomic solutions for the treatment case. These exchangeable applicators are hosted in such a manner that, by design, high voltage connections present minimised risk for operator or patient. An additional safety element is a so-called spacer, allowing an intrinsic operation within pre-defined conditions. New trends propose sensors to track treatment time, enable device quality control, and monitor environmental impact like the humidity to post-qualify the performed therapy.[421,464–466]

For a medical use case, innovative technologies first have to prove their benefits on the intended therapy in vitro before operating under in vivo conditions. Thus, to pre-qualify devices for future trials requiring clinical ethics approval as well as to validate safety and efficacy, a group of scientists in Germany, together with plasma device manufacturers, initiated a so-called pre-standard, the DIN SPEC 91315.[364,467] This German pre-standard proposes a set of in vitro studies designed specifically for plasma medical devices. Further refinement of the DIN SPEC 91315 in Germany is in progress, while internationally at other locations, i.e. Austria, Brazil, France, Korea, initiatives are also commencing to define electrical requirements for plasma devices.[468] With both the electrical safety definitions and the local regulatory medical device requirements (e.g. MDR for the EU), an overall set of qualifiers is defined. Although medical and regulatory requirements vary globally, these criteria have also been used for guidance evaluating new device innovations outside Europe, due to its investigative frontrunner availability and position.[469–474]

Further requirements need consideration if the treatment area has a local sensitivity on respective plasma components. Plasma is commonly described as a cocktail of reactive agents, namely emission in the UV and vacuum UV spectral range, a large variety of RONS, transient electrical fields, local temperature and charged species. The treated areas have to be considered individually on these components so that a treatment near the nose should consider the local reactive species exposure with a direct pathway into the lung. Similarly, the vestibular organ is known to react to magnetic fields from nuclear magnetic resonance imaging via impacts on the local fluids and ions, thus highlighting a careful electrical safety consideration for plasma sources, as it would be covered within the electrical safety qualification.[475]

10.2 Available plasma medical sources for CAP therapy – device categories

Presently a variety of plasma medical sources are on the market, following different technological pathways to provide

safe and efficient operation. When presenting an overview of these device categories and their differences here, it should also be noted that the international community so far has not defined a clear set of characteristics for a plasma device to be accountable as a CAP source for medical therapy. As a minimal foundation for this field, an accountable performance and safety validation in accordance to the DIN SPEC 91315 is recommended.[364,467,476]

Among the different types of plasma medical sources, a total of two major categories summarises the discharge principle within – the DBD and the plasma torch. Understanding the difference between both different technical approaches also clarifies the treatment philosophy.

A DBD uses a dielectric barrier to inhibit current flow from the power supply into the conductive plasma discharge onto the patient. This allows a direct treatment of the patient with the plasma under safe conditions. By properly selecting the dielectric barrier, an effective capacitance acts by inhibiting a current transmission. A dielectric material is non-conductive by nature and thus avoids a current flow from the power unit to the patient. At the same time, it is able to store charges on its surface, ideal to enhance consecutive plasma ignitions. In the case of alternating voltage, charges can be collected onto the dielectric and emitted again to supply a discharge with a tolerable current flow. Once a current limitation is in place, a transition from a filamentary non-thermal plasma into a thermal equilibrium plasma will not occur. While a thermal plasma can easily reach several thousand degree celsius, a non-thermal plasma can be operated at tissue tolerable temperatures down to room temperature. The DBD is the most common plasma medical source approach, branching into three sub categories – the VDBD, the SDBD and the PJ.[19] Sometimes the PJ are put into a category of their own, for the dielectric is not always the only limiting building block, for an intrinsic current limitation can be established within the electrical circuit; this is especially relevant in the case of a device operation in close vicinity to the surface.

The plasma torch on the other side is categorised as a hot non-thermal plasma discharge. Yet, by considering an indirect treatment approach at an appropriate distance, the discharge arrangement can still provide a tissue tolerable temperature. In order to make this device-specific feature less dependent on experience of the user handling it, provided spacers of the devices provides an intrinsic ideal treatment distance. From the thermal discharge region, a recombining afterglow is emitted downstream, and the wound treatment performed underneath. No direct contact with the plasma region is required for the medical therapy.

An overview of four major discharge types is depicted in Figure 19. Overall technological solutions for CAP require a local electrical field enhancement to reach breakdown

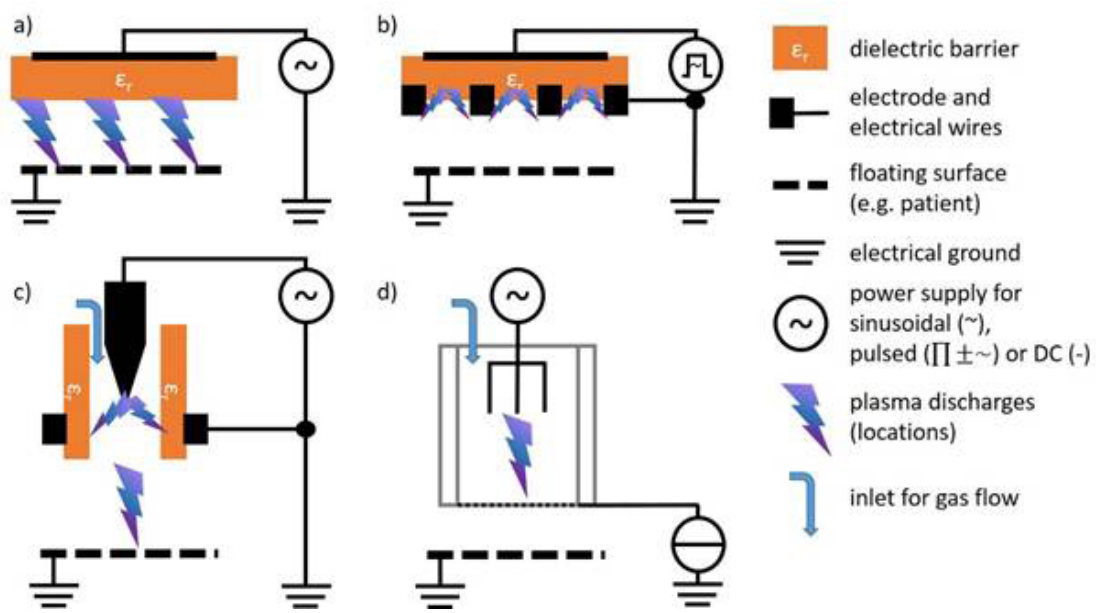

Figure 19: Electrical geometries for the four different discharge types.
a) VDBD; b) SDBD; c) PJ; and d) plasma torch

criteria. VDBD and SDBD are mostly operated in ambient air. Here, an electrical field of roughly 10kV/cm is required, meaning a high voltage of 10kV will ignite a discharge at a 1cm discharge gap. However, in the case of local electrical field enhancement by geometric shape adjustments, this simple ratio will not apply any more. For safety reasons, a minimal possible voltage is desired and reached by individual electrode geometries. In the case of DBD, the high voltage electrode is mostly placed behind a dielectric barrier to avoid direct contact with the electrode.

The placement of the grounded electrode defines the spreading of the discharge. DBD using ambient air and the treated target (e.g. a patient, the wound) as a counter electrode will ignite several plasma filaments, breaching the gap between dielectric and surface, and thus they are ignited into the enclosed volume (Figure 19a). The terminology VDBD therefore describes a discharge into open air between two electrodes.[19] The cocktail of reactive components is initiated within the whole gap and close to the targeted wound size, enhancing the complexity of the cocktail acting onto it. The filaments will be ignited stochastically over the extension of the high voltage electrode and, assuming an accumulation over a recommended timeframe, result in a large area treatment.

If the discharge device includes a grounded grid electrode on top of the dielectric barrier, the discharge is limited on the surface and will not reach into the volume (Figure 19b). These type of discharges are called SDBD.[19] Since a treatment surface such as the patient's skin is not required here for proper operation, they are also not sensitive to the distance towards the wound surface. This can provide advantages in the case of uneven wound surface. Yet the so-called "cocktail composition" (paragraph 10.1) reaching the surface is reduced with the treatment distance. Since the dose definition is still a topic of present research in plasma medicine considering the composition of the cocktail components, neither an advantage nor disadvantage can be stated. Here the qualification via a pre-defined set of measurements allows potential users a comparative data set for intended therapy.[477] Recent datasets showed clear decontamination efficacy.

Besides VDBD and SDBD operating in ambient air, the PJ systems require an active gas flow, supplying rare gases for more convenient discharge ignition.[19] The gas flushes in between the discharge system of a high voltage electrode, a dielectric barrier and an outer grounded electrode. The plasma is ignited inside the capillary, while the electrical field and the gas composition guide the discharge also into the ambient air. There, a mixture of working gas like Ar or He and ambient open air evolves and a variety of reactive species is generated. In special cases, even a temperature gradient can be initiated by the gas flow itself.[474] Furthermore, the operation within a rare gas enhances the overall visibility of this discharge due to emission in the visible spectral range. The generated cocktail composition has a similar complexity to the VDBD with discharges onto the surface, yet, in the case of PJ, the distance is of similar importance. Recent findings show a strong dependence of the distance to the surface like the wound area on the discharge performance, indicating a mode shift for the device controlled by the distance.[478] The so-called conductive and free mode differ in the sense that the free mode is limited to individual discharge filaments per voltage period travelling into ambient air, while in conductive mode the filament connects with the surface and a consecutive discharge initiate a secondary discharge onto the surface in an infrequent manner.

While the device types VDBD, SDBD and PJ operate either at sinusoidal, pulsed or mixed voltage at frequencies up to 26MHz, the plasma torch is a microwave powered system at 2.45GHz. Plasma ignition is initiated inside the device and, due to the high frequency and high distance to the surface, the discharge is not transmitted onto the wound directly. Since each device is associated with different compositions of cocktail components, a selection of the device to be used should be focused on the requirement for the desired therapy. These, however, are not solely limited on efficacy consideration, but also on ergonomic requirements or, in some healthcare sectors, on economic requirements like treatment time per patient instead of per area element. Other comparative perspectives might also be the impact of environmental conditions. PJ showed that the impact of ambient temperature for instance is less significant compared to the working gas humidity,[120,479] yet requiring an external gas supply, while DBD are affected by certain range of humidity in the ambient air.[480,481]

10.3 Emerging plasma sources for CAP therapies and their fields of application

Out of these initial device types available to clinicians and patients in a limited amount of countries, ongoing research

has led to the development of new CAP devices, sources and types. Within this chapter, new innovative discharge trends like the transferred discharge devices and upscaling solutions are presented. Furthermore, different new fields of applications that required and inspired new CAP devices are briefly addressed.

Beside further investigations into established discharge concepts of CAP for medical therapy like SDBD, VDBD and PJ, one new concept grows more attention – the transferred discharge method (TDM). This approach decouples the power unit circuit from the discharge relevant for the treatment by an in-between additional discharge due to the elementary conductivity of plasma itself. A primary discharge is ignited in a somewhat closed environment, while a secondary discharge is then generated towards the patient. The applied potential to ignite the primary discharge is transmitted through a dielectric barrier or a conductive wire to the treatment area. The TDM can be established as a PJ or a DBD, sometimes a CAP device even provides both varieties (Figure 21).[469,482–486]

Present PJ-based approaches of TDM focus on endoscopic treatment solutions or on treatment of infected, hard to reach treatment spots like in the tracheostomy tube (Figure 20a).[271,469,483,487–491] The TDM intrinsically disconnects a possible continuous current transfer through the temporal limited primary discharge and hence the resulting secondary discharge is ignited but without a conductive connection to the power supply. Resulting investigations showed tolerable safety levels with proven efficacy based on a DIN SPEC 91315 characterisation in one system.[473,474]

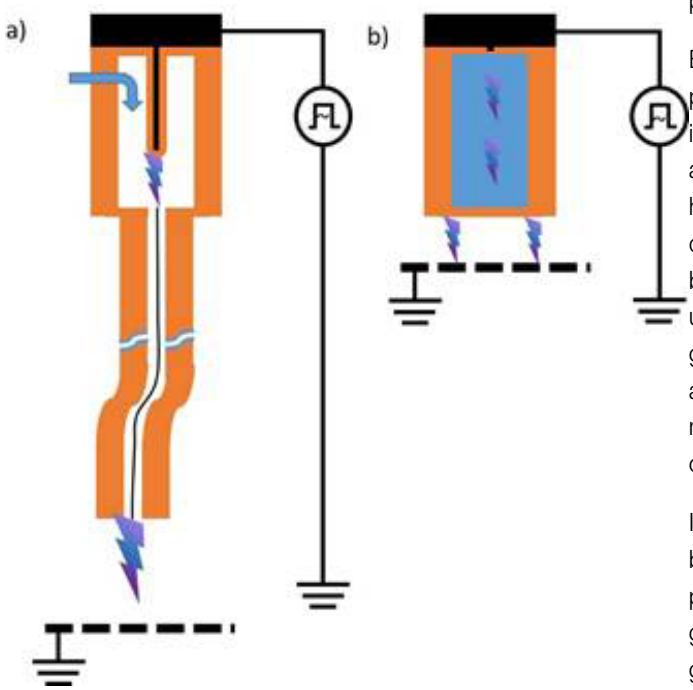

Figure 20: Scheme of transferred discharge systems found in the literature.

a) depicts a PJ based on the TDM; b) depicts a VDBD based on the TDM

Approaches based on VDBD combined with the TDM are not new but rather date back nearly a century.[492] However, given modern regulations and the advances in scientific validation of plasma therapies, an adapted innovation of this technology took place leading to a variety of devices.[469,484–486] Most devices operate by igniting the primary discharge inside a glass or quartz container. This container is previously vacuumed and filled to a low pressure level with a rare gas (Figure 20b). Here mostly neon is applied for its good breakdown criteria and possibly the visible emission colour. The secondary discharge outside the container is ignited inside ambient air and thus does not consume a constant gas flow. Also, it could in principle even generate discharge underneath a target if the tip is placed in full contact with a surface. Such systems are presently undergoing evaluations towards future therapy pathways in plasma medicine.[484,485]

Beside new discharge technologies, adaptations to previously presented technologies lead to new innovations in plasma medicine. One adaptation comes with the approach to upscale existing technologies. Based on the high complexity of the plasma technology, an upscaling does invoke several challenges that have to be solved before establishing a solution for a medical therapy. An upscaling allows the treatment of a larger area in one more generalised approach, while reducing treatment times and potential user burden. Such an upscaling is not just a matter of a multiplication factor, even full body dimensions could be considered.

In the case of DBD, the upscaling is commonly performed by exchanging the applicator and maintaining the same power supply unit.[11,187,477,493–495] Most concepts of a DBD geometry open a pre-designed upscaling solution,[480,481] given that the changing capacity would require adjustments in the power supply to sustain the electrical circuit. Continuous development of CAP therapeutic devices has led to new power sources which have the possibility to

adjust their inner resonant circuit to the outer resonant circuit via an integrated matching network.

In the case of PJ, upscaling is similarly approached with an adjustment of the design. Due to breakdown conditions requiring a high electrical field described as voltage over distance, an increase of capillary diameter would require an upscaling of the voltage. Thus the preferred approach is the multiplication of the discharge systems to the desired dimensions, commonly called a PJ array (Figure 21a).[274,494,496,497] While the treatment area can be increased, limitations on the ambient air admixture are induced, possibly altering the cocktail composition. One innovation aims to counteract this limitation by regulating the gas flow per channel and thus not invoking a drainage of ambient air.[498] Another solution was found when adjusting the tube length in an upscaled fashion of the remote ignition technology.[499] Considering new therapies, the variety of cocktail compositions possible with plasma devices is of special interest. Due to its complex nature, plasma can be generated in multiple different fashion, still hiding future potential.[471,498,500,501] With the increase of treatment area, the balance of operation time defined by safety thresholds such as reactive species, temperature, ozone and leakage current have to be balanced with the improvement of efficacy.

As an adaptation of PJ concepts, two further methods are under research, focusing either on adjustment of the flow shaping or on the reorientation of the discharge by controlling the electrical field (Figure 21c).[378,495,502–504] Shaping the gas flow results in a change of distribution of reactive species to a desired gradient on a surface based on fluid mechanics.[503] An additional effect is the removal of the flow impulse that is applied onto the wound surface in that concept. Yet this approach could reduce or extinct components from the cocktail.[504] The reorientation of the discharge generates a system in which the switch from the free jet to the conductive jet is controlled per design, while the enhanced discharge length along the ambient air generates an upscaled impact area on the surface.[378] Such technical requirements might have a slight negative impact on handling and ergonomic suitability for the desired therapy.

The innovations in CAP sources until this time focus on new and advanced discharge devices while not explicitly being focussed on a therapy in itself. Meanwhile, in some investigations, a new therapy indication is under development, highlighting the device for use in case-specific device design.

Among the therapy concepts under investigation, one will become the focus of solutions in the future – combination therapies. When plasma sources are tuned to be tissue tolerable and soft, they meet all safety requirements. Yet under these settings, the attribute of a mechanical impact on the surface, as known from surface treatment like etching, is lost, thus reducing its efficiency potentially. However, to treat diseases reaching a certain depth or being shielded by layers of, for example, biofilm, a mechanical pre-treatment is required. Such approaches are already in the clinical trial phase in dentistry in order to treat peri-implantitis.[471,505–507] Further combination therapies are frequently discussed

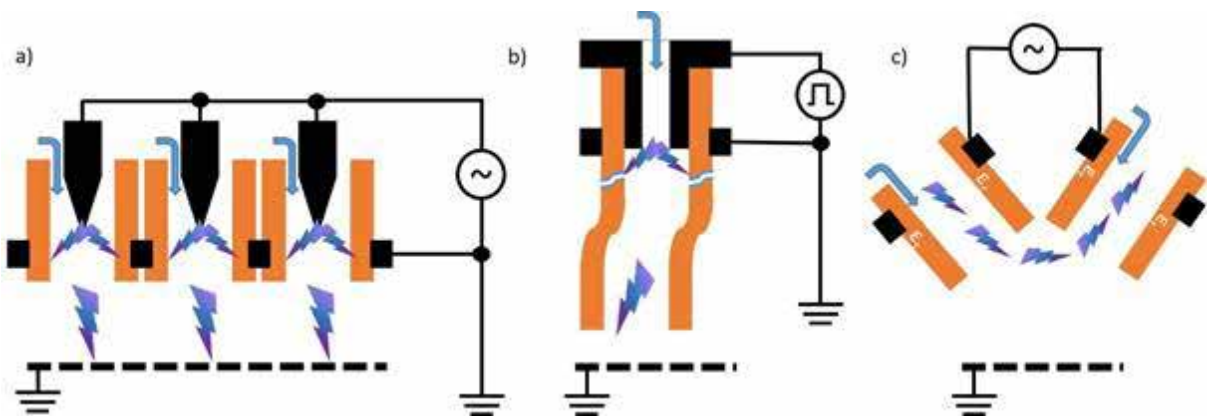

Figure 21: Innovative approaches to CAP solutions for medical therapy.

a) upscaling via a jet array; b) the plasma gun providing a remote ignition inside a capillary and a plasma propagation towards the tube exit; c) the V-jet igniting a discharge between two discharge systems.

at scientific events, showing the desire to broaden the field where it can be of help. Another known combination approach is together with pulsed electrical fields, known from the decontamination topics with plasma technology.[508]

As discussed in this Appendix, the potential of CAP innovations in plasma medicine is surely significant. Making these innovations reach the bedside of patients will, however, require dedicated multi-disciplinary effort and commitment from all parties involved directly or indirectly – researchers, HCPs, funding agencies and industry.

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