

REVIEW ARTICLE

Plasma applications in medicine with a special focus on dermatology

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Abstract

The recent tremendous progress in understanding physical plasma phenomenon, together with the development of new plasma sources has put growing focus on the application of plasmas in health care. Active plasma components, such as molecules, atoms, ions, electrons and photons, reactive species, ultraviolet radiation, optical and infrared emission and heat have the ability of activating, controlling and catalysing reactions and complex biochemical procedures. Thermal and non-thermal (i.e. cold) plasmas – both already widely established in medicine – are used for various therapeutic applications. Particularly in dermatology, plasma applications hold big potential, for example, in wound healing, such as efficient disinfection or sterilization, therapy of various skin infections or tissue regeneration. This review gives an overview on potential plasma applications in medicine – including the recent research on skin diseases – and summarizes possible interactions between plasmas and living tissue.

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Conflicts of interest

Some authors are designated inventors of various patents of plasma applicators and corresponding methods.

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Introduction

In physical sciences, plasmas are described as the fourth state of matter in addition to solids, liquids and gases. When mentioned for the first time in 1879 by the British chemist and physicist Sir William Crookes, this state was described as ‘radiant matter’.¹ Irving Langmuir introduced the term plasma in 1928 because the composition of the strongly ionized gas reminded him of blood plasma. The literal translation of the underlying Greek word ‘plassein’ is ‘to mould’.

Natural plasmas are estimated to account for more than 99% of the visible universe including the sun and stars, the solar corona, solar (stellar) winds, HII nebulae, the earth’s ionosphere, as well as

well-known phenomenon on earth, such as lightning and aurora borealis.

Plasmas are also artificially produced to be used, for instance, in displays and fluorescent lamps and for surface treatment of solid matter and, lately, also in the (bio)medical sector. In the past years, research has made tremendous progress in this area; at present, a number of different atmospheric plasma sources are available for application.

In early plasma applications, the thermal energy of plasma, i.e. heat and high temperature, was responsible for the desired effects: Thermal plasmas (>80 °C) have been used for tissue removal and destruction, cutting, cauterization, sterilization of thermally stable medical instruments and recently even for cosmetic tissue re-structuring.^{2–6}

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Current research mainly focuses on the non-thermal effects of plasmas: applications below the threshold of thermal damage (slightly above room temperature) aim at inducing a specific response or chemical modification by generating active species that are either produced in the plasma or in the tissue brought into contact with plasma. Non-thermal plasmas have only little effect on the surrounding (healthy) tissue (at relatively low room temperatures), but allow efficient disinfection and sterilization of living tissue within seconds by inactivating gram-negative and gram-positive bacteria, fungi, virus, spores, various parasites and foreign organisms or pathogens. Furthermore, plasmas allow controlled, high-precision tissue removal without inflammation or damage, modify tissues at the cellular level and avoid inflammation and scarring. An advantage of its gaseous form is the possibility to penetrate even in inhomogeneous surfaces, cavities and fissures down to the micrometre scale, at which traditional fluid or chemical techniques fail. Another large benefit of plasma treatment is the contactless, self-sterilizing, pain-free, non-invasive and pure physical application, which offers the possibility of drug delivery at the molecular level.

In early applications, non-living surfaces were treated with plasmas to obtain the desired effects in biomedical research. For more than 5 years now, surfaces of medical equipment have been routinely and successfully decontaminated with non-thermal atmospheric pressure plasmas (CAPs = cold atmospheric plasmas). Thermally unstable and inhomogeneous surfaces are difficult to clean with chemicals or heat and may even be destroyed; thus, plasmas provide an important and welcome alternative. This alternative is particularly important in times in which propagation of multi-resistant bacteria represents a growing global problem⁷ and, simultaneously, the effectiveness of antibiotic treatments diminishes. New strategies have to be found to face these problems. CAPs, as a 'physical' method, could offer a simple, fast, effective and economic way of disinfection of equipment, surfaces and people – a method that is unlikely to cause resistance or allergic and toxic reactions. Although the bactericidal effect of plasmas is undisputed, most of the mechanisms of action are still unknown and need to be investigated. So far, only few researchers have conducted systematic investigations of the interaction of plasma and microorganisms, living tissues, and – in particular – applications in medicine.

After the first *in-vitro* studies on various animal and human cells, the application of CAP on live tissue remained only a question of time. A broad spectrum of medical applications in health-care, in particular the bactericidal and bacteriostatic properties of cold plasmas, have paved the way for analyses on human living tissue and patients.

This review concentrates on various plasma applications in medicine, particularly in dermatology.

Physical background

For the use of plasmas in many applications, plasmas consist of a partially ionized gas, which contains free charge carriers, i.e. ions

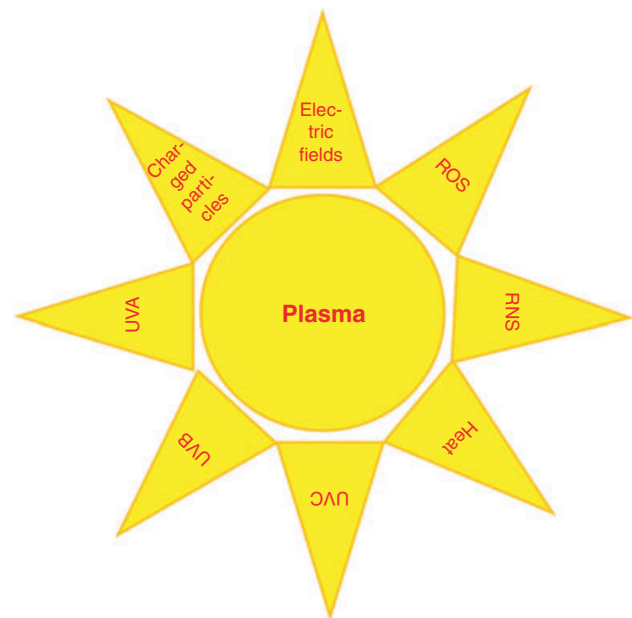


Figure 1 Components of plasma. ROS, reactive oxygen species (O, OH, H₂O₂, O₃); RNS, reactive nitrogen species (NO, NO₂).

and electrons that are neither bound to atoms or molecules nor to active radicals, excited molecules or neutral atoms/molecules (Fig. 1). Plasma is generated if sufficient energy is applied into volume to separate electrons from atoms and molecules. The ability of positive and negative charges to move freely is responsible for the high electrical conductivity and, as a consequence, for the strong response to electromagnetic fields.

Plasmas, which can exist in a variety of states, can be classified into low-pressure plasmas, atmospheric-pressure plasmas and high-pressure plasmas. A further necessary differentiation between thermal and non-thermal (= non-equilibrium) plasmas is based on the relative temperatures of electrons, ions and neutrals. In 'thermal plasmas', all the temperatures of electrons, ions and neutrals are same, so that the gas temperature is normally very high. Applicability is limited because of the very high temperature and consecutive cooling requirements. In 'non-thermal plasmas', due to energy consumption and geometry of device, only smaller electrons are heated and are consequently at a higher temperature level than ions and uncharged molecules. The difference in mass is enormous so that electrons may be at several thousands to several ten thousands degrees Celsius, whereas the entire plasma remains at almost room temperature. CAPs thus allow applications at low temperatures without any particular extra cooling and can be used to treat heat-sensitive objects or matter. CAPs are characterized by high excitation selectivity and non-equilibrium chemical reactions. Most research in medicine now focuses on the application of CAPs; this application is particularly promising because of the

Table 1 Types of cold atmospheric-pressure plasmas (CAPs)

Plasma-type	Examples for the technology used	Production technique and properties
Direct plasmas	Dielectric barrier discharge plasma source	Skin or other tissues are used as an electrode, the current produced passes through the body.
Indirect plasmas	Plasma needle, plasma torch	Plasma is produced between two electrodes and then transported to the area of application entrained in a gas flow.
Hybrid plasmas	Barrier coronal discharges	Combines production technique of direct plasmas with properties of indirect plasmas. A grounded mesh electrode is introduced with much smaller electrical resistance than the tissue, thus the current passes through the wire mesh.

ability of CAPs (in principle, at least) to initiate, push, control and catalyse complex biochemical reactions and procedures.

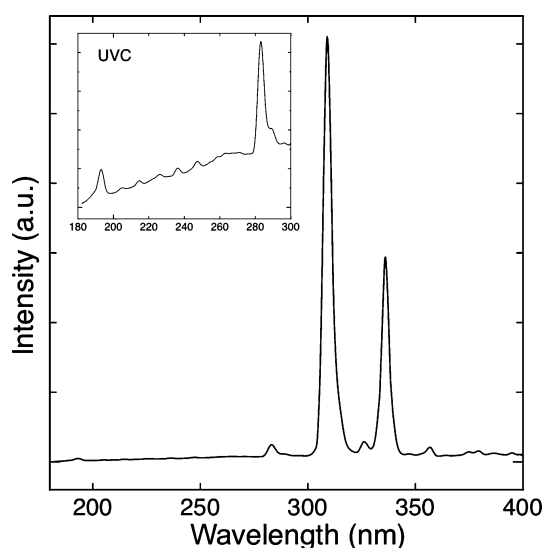
Relevant parameters of (medical) plasmas are electron temperature, electron or ion density, ultraviolet radiation (UVR, i.e. UV A, B, C), density of free radicals, temperature of neutral gas, gas flow and composition and optical and infrared emission. An important factor for the effect of plasma is the flux of active charged (electrons as well as positive and negative ions such as Ar^+ and N_2^+) and uncharged species of atoms and molecules generated by non-equilibrium plasma reactions (like O_3 , OH, H_2O_2 , NO, OH radicals etc.) on the surface of living tissue. Depending on the desired effects, active agents can – to some degree – be ‘designed’ into plasma to produce a ‘chemical cocktail’, which is then delivered to a respective target.⁸

Three types of CAPs are to be differentiated: direct, indirect and ‘hybrid plasmas’. In direct plasma treatment, in which tissue represents one of the plasma electrodes, various active uncharged atoms and molecules flow to and through the tissue surface, in addition to UV radiation and, most importantly, also in addition to charges such as electrons and positive and negative ions.⁹ Or, in other words, current passes through the body. The plasma device and the tissue surface need to be constantly at the same close

(~1 mm) distance. The most widely used technology is the ‘dielectric barrier discharge’ (DBD) plasma source.¹⁰ Indirect plasmas are produced between two electrodes and then transported to the desired area via gas flow so that the body itself is not needed as a plasma electrode.^{11,12} Indirect plasmas are preferable in ‘rough’ and large treatment surfaces. Active species are typically delivered via gas flow or diffusion, which is a very fast method. Furthermore, additional effects of the admixture of different gases can be used. ‘Hybrid plasmas’, also called ‘barrier corona discharges’, combine the production of direct plasmas with the essential current-free property of indirect plasmas by introducing a grounded mesh electrode through which the current passes (Table 1).

Plasmas can be produced by discharges in noble gases, in air or in other mixtures. No general requirements exist for possible plasma components. Medical applications need high safety regulations to prevent potentially damaging or toxic side-effects. Regulations for plasma sources refer to electrical safety, production of UV and reactive species and the limitation of current through skin. According to the WHO guidelines (ICNIRP), UVR exposure of unprotected skin should generally not exceed 30 J/m^2 in the spectral region of 180 to 400 nm¹³ (Fig. 2, Table 2). The upper ozone limit is 50 ppb (see CPSC Consumer Products Safety Commission report of 26 September 2006). For nitrogen species, the US National Institute for Occupational Safety and Health (NIOSH) recommends an exposure limit of 5 ppm for NO_2 and 25 ppm for NO over an 8 h period.

Cold atmospheric plasmas typically deliver up to 10^9 to 10^{10} active agents per cm^2 and second. During a typical (10 to 100 s) application, 10^{10} to 10^{12} active molecules/ cm^2 are generated, approximately the amount of molecules found in a typical lotion with 0.1% to 1% concentration of active agents (2.5×10^{10} to

**Figure 2** UV-spectrum of MicroPlaSter β .**Table 2** Comparison of UVR-intensities (over 5 min) of natural sun measured within 1 year in Garching/Germany and MicroPlaSter β (microwave power 100 W, main (Ar) gas flow rate 2.5 slm, measured 20 mm apart from the torch's opening)

	MicroPlaSter β (5 min)	Natural sunlight (5 min)
UVA (320–400 nm)	<100 $\mu\text{W}/\text{cm}^2$	600 $\mu\text{W}/\text{cm}^2$
UVB (280–320 nm)	40–60 $\mu\text{W}/\text{cm}^2$	30–50 $\mu\text{W}/\text{cm}^2$
UVC (180–280 nm)	10–16 $\mu\text{W}/\text{cm}^2$	1–2.5 $\mu\text{W}/\text{cm}^2$

2.5×10^{11}). Of course, 'plasma delivery' occurs at an atomic or molecular level; in lotions and ointments, the active components have to be immersed inside a carrier medium.

Influence of plasma on living cells: plasma-tissue interaction

Several studies have been initiated to identify possible adverse effects of plasma applications.

The factor 'UVR' in particular deserves special attention. Electric discharges in gases provide polychromatic UVR. Biological effects strongly depend on the electromagnetic spectrum of UVR. Short-wavelength UVC (100 to 280 nm) emitted by the sun, which is almost completely absorbed by the atmosphere, is the most cytotoxic type because of its possible induction of mutagenesis and photochemical oxidation processes in cells. Two main mechanisms are responsible for the UV-induced cellular damage at the molecular level: direct effects based on UV energy absorption by cellular macromolecules and alterations of DNA, and proteins and lipids caused by UV-induced oxidative stress.

Spectral characteristics of plasma-generated UVR can vary, depending on the emission spectrum of input gas(es) and their purity as well as on the addition of different gases to the main input gas.¹⁴ UVR doses depend on the plasma device, the required exposure time, the type of discharge gas and the gas composition. Nevertheless, spectral composition and intensity of plasma-generated UVR are critical parameters for the duration of plasma irradiation and intensity in human tissue;¹⁵ thus, plasma devices must be carefully calibrated. To extend the flexibility of plasma therapy, plasmas can be designed towards reduced UVR-content and elevated density of reactive species.

Sosnin and colleagues showed in their investigations on *E. coli* and CHO-K1 (Chinese hamster ovarian cells) that UVR could lead to cell death in fibroblasts, but only at much higher doses than in bacteria.¹⁶ In combination with active oxygen radicals, the UVR dose sufficient to kill cells was lowered.¹⁷ Kalghatgi *et al.* investigated plasma-induced DNA damage by measuring double strand breaks (DSB) by means of immunofluorescence and western blots.¹⁸ Short (5 s) plasma treatment at a low power of 0.1 W/cm^2 resulted in DSB in mammalian cells. The level of DNA damage depended on the plasma dose: at low levels of $<1 \text{ J/cm}^2$, the damage was reversible, whereas higher doses resulted in cell apoptosis.

In experiments on cadaver tissue, Fridman and colleagues found that treatment with FE-DBD (Floating Electrode–Dielectric Barrier Discharge) plasma for up to 5 min did not show any macroscopic or microscopic changes. After 10 min treatment, vacuolization of keratinocytes was detected, both in dead skin and *in vitro*. At an application time of 2 min, which is sufficient for significant bacterial load reduction, no consequences to blood or tissue were registered.¹⁹ In a phase I study, we neither detected any histological changes in *ex vivo* human skin after 7 min of plasma treatment (Fig. 3a, b) nor in images of Atomic Force Microscopy of human skin and HeLa cells after 4 min of plasma

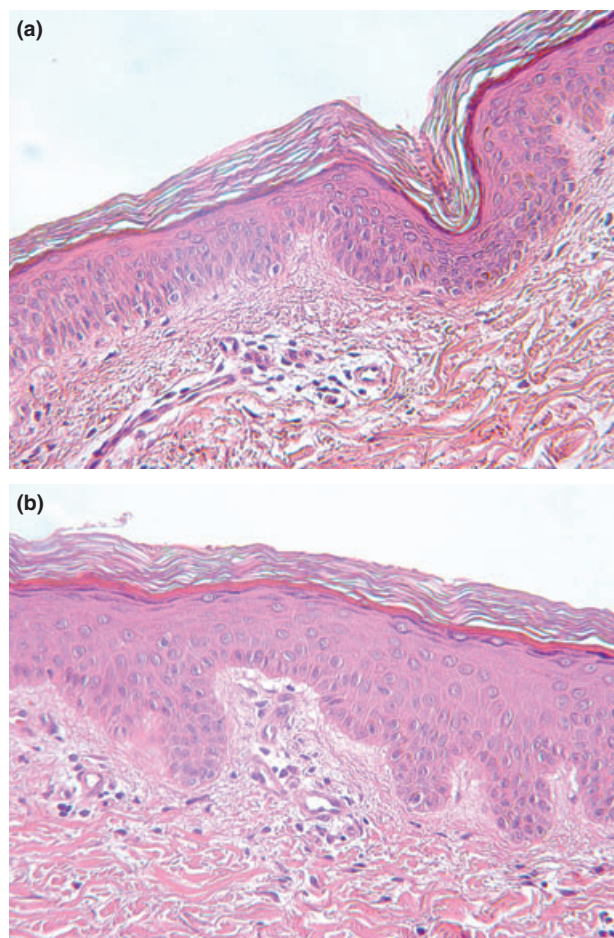


Figure 3 (a) Histology (H&E staining, 20 \times) of normal human skin (*ex vivo*) treated for 3 min with low-temperature argon plasma (MicroPlaSter). (b) Histology (H&E staining, 20 \times) of normal human skin (*ex vivo*) treated for 7 min with low-temperature argon plasma (MicroPlaSter). No detectable histological changes of healthy skin.

treatment. Additionally, we did not detect changes in blood counts or microscopic changes of erythrocytes after 10 min of exposure, whereas 4 min of plasma treatment destroyed the cell structures of *Escherichia coli*.

Daeschlein and co-workers examined the influence of plasma treatment on skin up to a depth of max. 250 μm by means of *in vivo* confocal laser scanning microscopy (CLSM).²⁰ This method allows surveilling the cell integrity of the epidermal layers and even affects on deeper structures such as papillar bodies, vessels and follicles. Daeschlein and co-workers treated the epidermis of human legs, palms, fingers and soles with plasma (produced by an atmospheric pressure argon jet over 3 min) as an antibacterial therapy. No microscopically detectable changes induced by plasma treatment were registered compared with untreated skin before treatment and 5 min, 60 min, 2, 6 and 14 days after one single treatment.

Kalhatgi and co-workers showed that CAP application at short exposure times down to 30 s induced proliferation of endothelial cells,²¹ whereas treatment at longer exposure times (>60 s) induced apoptosis. Cells treated with CAP for 30 s showed twice as much proliferation as untreated cells. Responsible for this proliferative effect could be the release of growth factors such as fibroblast growth factor-2 (FGF2) induced by plasma.²² Wende and colleagues showed in an *in vitro* wound model (scratch assay) of human keratinocytes (HaCaT) that these cells closed a mechanically generated gap after 40 s of plasma treatment (argon plasma jet) with a rate of 1 mm/day compared with 1.1 mm/day without plasma treatment. Longer treatment time resulted in cell detachment. On the other hand, bacterial growth was reduced in a scratch assay with added *Escherichia coli* bacteria (10^4), whereas HaCaT cells survived. Reduction in the gap was accelerated in treated and infected controls compared with an untreated but infected cell system.²³ Tipa *et al.* also showed by using a cold atmospheric plasma needle (13.56 MHz micro-jet in helium) to treat cultured 3T3 fibroblast cells, effects on fibroblasts depend on time and dosage; in a second design, the model of a real wound was created.²⁴

In summary, the results of the different studies on the effects of CAPs on mammalian cells so far have shown that effects depend on dosage and time: The application of plasma can cause necrosis, apoptosis or cell detachment.

In contrast to the evident effect on bacteria, living cells (human blood cells or skin tissue) are not damaged at parameters needed for sterilization.^{25,26} Dobrynin *et al.* deduced the following three hypotheses from experimental data with human cadaver tissue, living mice, pigs and cell cultures of different mammalian cells:²⁷ (i) Eukaryotic cells are protected from ROS because of their different cell metabolism. (ii) Higher order organisms of eukaryotic cells result in better resistance mechanisms to external stress. (iii) Prokaryotic cells have a much higher surface to volume ratio so that a lower dose of 'poison' is required for inactivation.

Induction of apoptosis represents a crucial issue in cancer treatment because cancer cells are often able to inhibit apoptosis and, therefore, are more resistant to chemotherapeutic drugs. Fridman *et al.* showed in an *in vitro* study that complex biochemical processes lead to programmed cell death of melanoma cells (ATCC A2058). Apoptosis may occur hours and even days after plasma treatment with FE-DBD at significantly lower doses than those required for cell necrosis.²⁸ It could be shown that plasma has a direct effect on cells; apoptosis was not induced by 'poisoning' the growth media or by interaction with aluminium dishes. Reactive oxygen species that penetrate cells and induce DNA damage seem to be responsible for inducing apoptosis in melanoma cells. On the other hand, apoptosis was significantly decreased by pre-treatment with *N*-acetylcysteine, a free radical scavenger.²⁹ Zucker *et al.* used a plasma needle (80 to 120 kHz, <1 W, Helium flow rate from 3 to 9.7 L/min) to treat melanoma cells including premetastatic (WM793B) and metastatic (1205Lu) cell lines

derived from lung metastasis of WM793B cells in nude mice compared with normal human primary keratinocytes (HEK), both in co-cultures and separate cultures.³⁰ Compared with HEK, premetastatic and metastatic melanoma cells showed a highly significant destruction rate and an increased percentage of cell death (detected by TUNEL assays). Further studies and characterization of the mechanisms of (programmed) cell death should be initiated to improve our comprehension of the interactions of CAP with living cells.

Decontamination of inanimate surfaces and living tissue by CAP

Various studies have shown the efficacy of CAP against gram-positive and gram-negative bacteria^{12,31–36}, biofilm-producing bacteria^{35,37–39}, virus, fungi and spores.^{36,40,41}

The advantages of sterilization by CAPs – which have been used since 1996 for inactivating bacteria⁴² – are penetration into small cavities, high quality of sterilization, the high speed of this method and the relative independence of the surface properties of the sterilized objects. In that way, application to heat-sensitive and chemically reactive material is facilitated. Different factors responsible for the sterilization or disinfection by plasmas are discussed: charged particles, reactive oxygen and nitrogen species and UV photons (physical mechanisms) as well as biological mechanisms (cellular processes) – initiated by the influence of plasma – are supposed to damage cell membranes and the DNA.^{12,32,38,42–45}

In a phase I study with an indirect non-thermal plasma source at atmospheric pressure [MicroPlaSter, 6 electrode torch, 110 W microwave power, argon gas (Fig. 4)], we obtained a 10^6 reduction in the bacterial load in treated agar plates (different gram-positive and gram-negative bacteria) (Fig. 5). The reduction persisted during subsequent incubation for at least 2 days. In

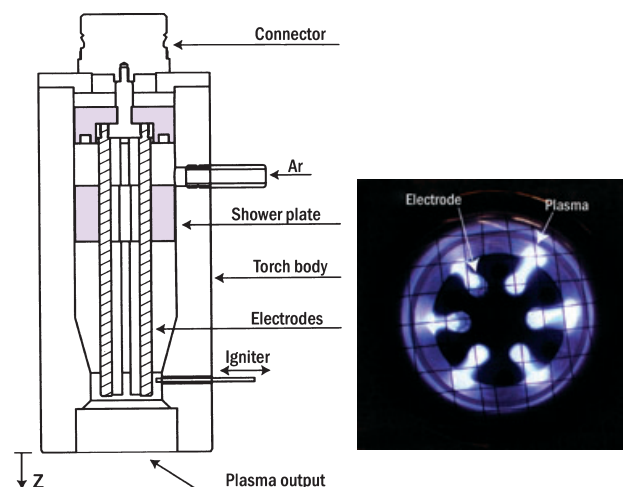


Figure 4 The plasma torch. Microwave (2.45 GHz, 86 W). Ar (2.2 slm). Torch body is cooled by airflow.

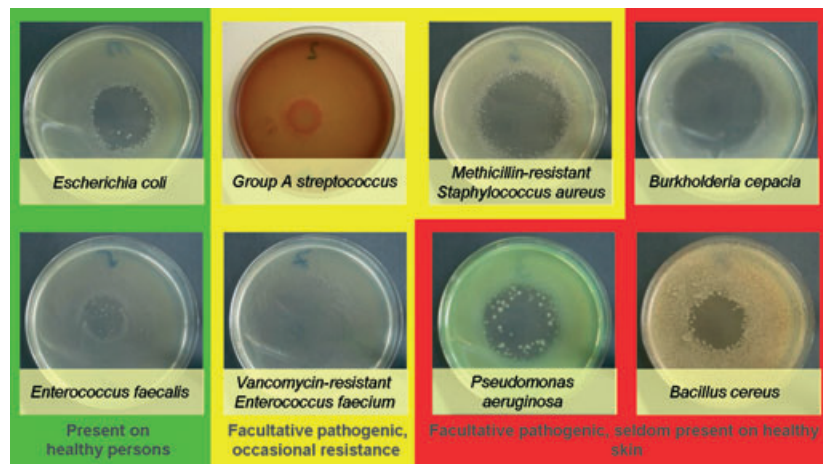


Figure 5 Efficiency of 2 min of plasma treatment (plasma torch) against different bacteria relevant in wound healing.

further tests, the (argon) plasma proved to be highly effective against different – even multi-resistant – bacteria (gram-positive and gram-negative) and yeasts, for example *Candida albicans*.^{11,19} Recent results of our group have indicated that bacteria have neither primary nor secondary resistance to plasma.

These studies still leave a number of issues open for discussion, for example the ‘choice’ and design of the plasma type and device, metabolic changes in the surviving organisms⁴⁶ and inconsistent information about the influence of UVR on bacteria inactivation efficiency.^{25,47}

Furthermore, gas discharges of dimensions in the range from micrometres to millimetres – termed ‘microplasmas’ – can operate at atmospheric pressure, offering new medical and industrial applications as air purification (decontamination of waste gas streams and destruction of volatile organic compounds⁴⁸) and bio-waste management. The list of potential applications, in the medical sector, grows continuously: from sterilizable air filters to air sterilization in operating rooms to sterilize surfaces in health-care, for example of medical instruments or in food processing.^{9,49–52} Even removing proteins from surfaces of medical instruments is possible, probably because of the influence of reactive oxygen species; reductions of up to 4.5-log are possible.^{53,54} Lee *et al.* showed the dissolution or removal of biofilms (produced by both gram-negative and gram-positive bacteria) in less than 20 s. Growth of planktonic bacteria could be stopped within 5 s.⁵⁵

In addition, distilled water, treated for 5 min by non-thermal quenched plasma, showed significant microbial disinfection properties to inactivate planktonic and adherent cells of various bacteria and could therefore be a new approach to treat contaminated matter.⁵⁶

In summary, new options arise in the prophylaxis and therapy of diverse skin diseases triggered by pathogenic germs. Bactericidal and fungicidal effects are observed even through fibrous textiles. Biofilm destruction by CAP could be used for the sterilization of

medical implants and catheters as well as in dentistry. In future, new plasma sources, such as HandPlaSter, a hybrid cold atmospheric plasma source for hand disinfection within a few seconds ($<0.5 \text{ W/cm}^2$, 18 kV_{pp}, 12.5 kHz), could play an important role in fighting nosocomial and community-acquired infections as well as multi-resistant bacteria.

Medical applications of plasma: ‘plasma medicine’

Blood coagulation

High-temperature plasma, applied in a non-contact manner without smoke production, vaporization or carbonization, has already been used for several years in nearly every kind of surgical specialty in the form of cauterization devices (Argon-Plasma Coagulation, APC).^{57–61} In newer applications, blood coagulation without thermal effects and risk of bacterial contamination and without any negative effect on the surrounding tissue is achieved by application of CAPs and by stimulation of specific natural processes in blood coagulation.^{62–64} Extensive platelet activation and aggregation as well as fibrin formation are observed after plasma treatment. Moreover, plasma treatment may also significantly change protein concentrations, clotting factors, pH and ionic strength in blood plasma – even in anticoagulated plasma.⁹

Effects of CAP on wound healing

Skin diseases caused by bacteria are one of the main reasons for the hospitalization of patients, costing billions of Euros each year. A very common affliction is infected chronic ulcers of the lower leg. With a prevalence of approximately 1% of the population in developed countries,⁶⁵ infected wounds account for an estimated 1–2% of annual health care budgets. Furthermore, infected wounds represent a major reservoir for multi-resistant bacterial strains. Global health care associations consider multi-resistant

bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) in particular, as a global threat.^{7,66}

Standard treatments of infected wounds include topical and systemic antibacterial regimens; however, these regimens are often limited by the development of bacterial resistance to antibiotics or by allergic skin reactions. Plasmas can be easily applied to wounds because of their temperature just slightly above body temperature and the manner of application, i.e. a contact-free application for 'rough' surfaces down to the micrometre scale. Apart from the efficacy in killing bacteria, plasma treatment of infected wounds does not seem to have side-effects such as bacterial resistance or allergic skin reactions.

Infections are known to impair wound healing; thus, reducing the bacterial load in chronic wounds should also improve wound healing itself. In 1970, Robson *et al.* concluded that bacterial loads larger than 10^5 CFU/tissue correspond to wound infection.⁶⁷ Moreover, the type of microorganism plays an important role, for instance much lower loads than 10^5 CFU/tissue of β -haemolytic Streptococci, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are sufficient to impair wound healing. Trengrove and colleagues found significantly delayed wound healing if more than four species of bacteria were present in a wound.⁶⁸ Cell culture studies have shown that plasma treatment may modulate wound healing not only by reducing the bacterial load but also by directly influencing the biological behaviour of epidermal and dermal cells.^{22,23}

After establishing the optimal bactericidal dosage and satisfying the safety requirements stated by the medical devices directive and the ethics committee, we started a phase II clinical trial. Up to now, more than 150 patients with chronic infected and colonized wounds or ulcers have been treated with indirect low-temperature argon plasma (MicroPlaSter) (Fig. 6). Once a day, patients received standard wound care and, as an 'add on' therapy, 2 to 5 min plasma treatment on randomized wound(s). Prior to the first plasma therapy, a high-pressure water jet or a scalpel was used to clean the wounds of debris. Before and after plasma treatment, standard bacterial swabs or smears of nitrocellulose filters were taken from plasma-treated and non-plasma-treated areas. These filters were placed on agar plates and then incubated for 12 h. Digital images allowed computer-assisted calculation of the bacterial load before and after plasma treatment. Possible side-effects such as pain were documented according to a standardized WHO score ranging from 0 to 10.

After well over 1300 treatments of 150 patients (on average 9.2 treatments per patient), no side-effects have occurred so far, and treatment is well-tolerated by almost all patients (Fig. 7a,b and c). As standard smears turned out to fail in accuracy and reproducibility in detecting changes in the bacterial load, we determined the type of bacteria by means of the swab technique once per week. On the other days, we used nitrocellulose filters, which yielded much more accurate and self-consistent results. In an interim analysis of 291 treatments in 36 patients, we found a highly significant (34%, $P < 10^{-6}$) reduction in bacterial load in



Figure 6 Treatment of a chronic leg ulcer with the plasma torch.

wounds treated with argon plasma compared with untreated wounds.⁶⁹ This reduction was found in all types of bacteria, even in multi-resistant bacteria such as MRSA, as shown in the preceding phase I study.

In an uncontrolled study with the system 'Plasmafon', Fetykov *et al.* observed two times faster complete wound healing (20th day) in 48 patients with diabetic foot disease. The authors also observed a decrease in pain within 5 days as well as beginning epithelization at the borders after 10 days. However, the authors did not define the exact composition – in particular the UV-spectra – of the cold plasma applied.⁷⁰

Advances in tissue regeneration rely on nitric oxide (NO), which also seems to play a key role in wound healing. It is possible to generate exogenic NO in plasma. By applying medical air-plasma devices, high concentrations of NO can be provided that stimulate regenerative processes.⁷¹ Wounds closed significantly faster in experimental animals than in the control group (the mean time to complete healing was 7.5 days shorter = 24.6%). Interestingly, bacterial load was only observed in the control group. Endothelial destruction, microthrombosis and erythrocyte slugging were less expressed in treated animals. Furthermore, wounds consciously contaminated with *Staphylococcus aureus* had a shorter wound healing time by 31.6%.

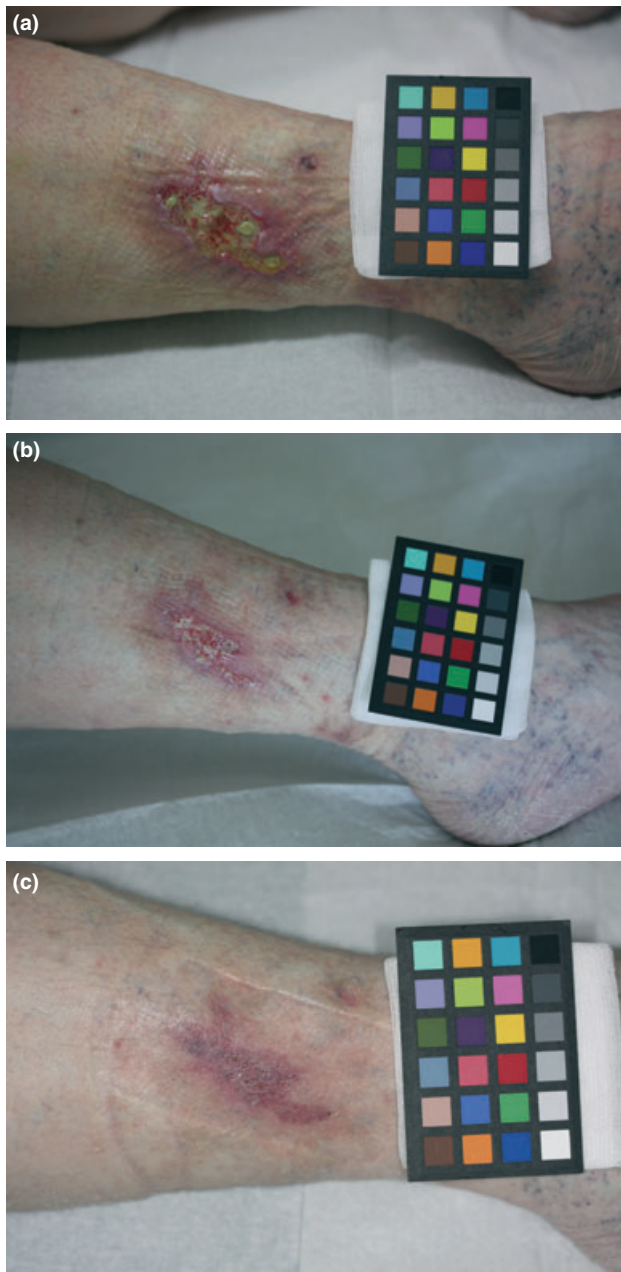


Figure 7 Inflamed ulcer of the right lower leg before treatment (a), after 12 (b) and 20 treatments (c) (5×/week) with cold-atmospheric argon plasma generated by MicroPlaSter β. Reduction in bacterial count and immunomodulatory effects of argon plasma therapy could result in a faster closure of chronic ulcers.

Possible mechanisms of NO for enhancing wound healing are, amongst others, vasodilatation and normalized microcirculation, direct bactericidal effect (because of formation of peroxynitrite), stimulation of bacterial phagocytosis, necrotic detritus by neutrophils and macrophages, inhibition of free oxygen radicals,

improvement of nerve conductance, regulation of immune-deficiencies, stimulation of fibroblast and vascular growth by secretion of cytokines, a direct effect on fibroblast proliferation, increased collagen synthesis and proliferation of keratinocytes.^{71,72} Shulutko *et al.* showed in an uncontrolled study of 65 diabetic patients with purulent and necrotic lesions of the lower extremities that all phases of the wound healing process were shortened under NO-therapy with a system called 'Plazon'. The epithelization rate doubled with continuous treatments. Inflammatory reactions and pain could be reduced, the period of inpatient treatment was significantly shorter and the amputation rate decreased.⁷³ Lipatov *et al.* noted acceleration of wound healing processes by NO-therapy in 40 patients with purulent wounds,⁷⁴ which was probably caused by significantly improved microcirculation in the wound zone. The 'Plazon' device has been approved for clinical use for 9 years.⁷⁵ During this time, this device has been successfully applied in tens of thousands of patients with different afflictions. However, no controlled studies are yet available.

Treatment of atopic eczema with CAP

Against the background of the fact that skin colonization with potentially pathogenic microorganisms can cause infections and can trigger different skin diseases (e.g. atopic eczema), Daeschlein *et al.* quantified the effect of CAP on bacterial skin colonization.⁷⁶ The skin of a patient with extended *Staphylococcus aureus* colonization was treated with a non-thermal atmospheric plasma jet (1–5 kV, 1.5 MHz, argon gas) over 3 min. The authors showed that *Staphylococcus aureus* was selectively eradicated in extensively colonized skin, whereas protective physiological flora (*Staphylococcus epidermidis*, *Staphylococcus haemolyticus*) was not modified but mobilized from deeper skin to the surface (monitoring by CLSM).

Mertens *et al.* used a DBD device (frequency 90–700 Hz, voltage 3–7.8 kV) to treat atopic dermatitis on the upper arm of a patient (1 min plasma application once daily for 30 days). The authors stated an antiseptic effect, abatement of skin reddening, as well as a significant reduction in pruritus for hours.^{17,77} Over a course of 2 days, *Staphylococcus aureus* colonies were reduced by more than tenfold. Application of CAPs may also present a novel treatment option for various other pruritic diseases (Fig. 8).

Plasma skin rejuvenation

In 2005, Plasma Skin Regeneration technology (PSR) was approved by the US Food and Drug Administration for the treatment of facial rhytids, superficial skin lesions, actinic keratoses, seborrheic keratoses and viral papilloma with different settings. Technically, PSR is a radio-frequency non-equilibrium thermal plasma jet with nitrogen as a carrier gas (1–4 J/pulse, 1–4 Hz), which directly delivers heat energy to tissue, independent of chromophores in contrast to selective photothermolysis of lasers.^{2,3} The rapidly cooled plasma causes controlled thermal damage to the skin, consequently stimulating new collagen production, reducing elastosis and improving photodamaged skin.



Figure 8 Treatment of a patient with pruritic disease (Prurigo nodularis Hyde) using argon plasma (MicroPlaSter β).

During this invasive treatment, patients need local or systemic anaesthetics. Results are comparable to carbon dioxide laser ablation, but plasma application has the advantage of causing minimal erythema and no pigmentary changes.⁷⁸ Histological studies confirmed collagen production and remodelling of dermal architecture in treatment areas.^{2,4,6,79}

Kilmer *et al.* showed average improvement of overall facial signs of skin ageing of 50% within 1 month with one single treatment at high energy (3–4 J).⁵ Potter *et al.* showed (with skin moulding in 11 patients) fine line wrinkles to be reduced by 24% within 6 months ($P = 0.005$) and abated acne scarring of 24% ($P = 0.001$) with PSR in the power range of 1–4 J.⁷⁸ Bogle *et al.* showed rather similar results, reducing facial rhytids by 37% within 3 months of full-face treatments, although they used three low energy treatments (1.2–1.8 J) in their study. Study participants even noted 68% of progress in facial appearance.

Positive effects have been observed for many other treatment conditions, such as dyschromias, photodamaged skin including non-facial sites such as hands, neck and chest,⁸⁰ skin laxity and acne or traumatic scars.^{4,81}

In combining nitrogen plasma skin regeneration with aesthetic facial surgery, Holcomb *et al.* enhanced outcomes for various procedures such as brow-lift, blepharoplasty and midface-lift, etc. without an increased risk of dermatological or surgical complications.⁸²

Other possible medical applications of plasma

Superficial bacterial skin infections such as erythrasma, impetigo contagiosa, folliculitis, gram-negative foot infection and ecthymata or fungal infections like tinea pedis or onychomycosis may be improved or healed by CAP application. As plasmas can penetrate textiles, even prophylactic treatments, for instance of tinea pedis, may be possible. Another example of an infectious skin disease is cutaneous Leishmaniasis, in particular post-Kalar-azar dermal Leishmaniasis. By treating *Leishmania* parasites (promastigotes of

Leishmania major) and human macrophage cultures, Friedman and colleagues found that inactivation of 20% to 30% of macrophages required 2 min vs. 20 s for inactivating 100% of promastigotes.⁸³ Promastigotes showed apoptosis-like behaviour similar to cancer cells. CAP could also become a treatment option in dental medicine: As plasmas are able to penetrate even into small, microscopic openings, they could be used for the prophylaxis and therapy of periodontal disease, one of the most common chronic oral diseases in the world,^{84,85} as well as for root canal infections⁸⁶ or chronic gingivitis.⁸⁷ A pleasant side-effect of plasma treatment is a 'teeth whitening' effect and the removal of tooth surface proteins.^{55,88,89}

Conclusion

This review described different aspects of the range of possible applications of plasmas in medicine – in particular in dermatology – without claiming completeness. Similar to any other emerging and dynamic area of research, there is still plenty of scope for further studies and new discoveries. Applications of plasmas will expand, in dermatology, and more and more possible indications will arise in the context of multi-disciplinary research. Feedback, collaboration and suggestions from physicists, biologists, chemists, engineers and physicians will facilitate further medical research. Fundamental cell biological tests, physical investigations and randomized studies with *in vivo* applications have to be undertaken to understand the different mechanisms of interaction between plasma and living cells and tissue and to 'design' plasmas for specific applications. This research must be supplemented with careful studies and possibly new standards for dosages, composition and electromagnetic safety requirements. A new technology is being created for a wide range of medical applications, from prophylaxis to regenerative medicine to treatment. At present, it is still difficult to predict all treatment options in which plasmas may be involved. In any case, non-thermal plasmas appear to be one of the most promising and expanding fields of research in medicine.

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