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Plasma medicine: an introductory review

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Abstract. This introductory review on plasma health care is intended to provide the interested reader with a summary of the current status of this emerging field, its scope, and its broad interdisciplinary approach, ranging from plasma physics, chemistry and technology, to microbiology, biochemistry, biophysics, medicine and hygiene. Apart from the basic plasma processes and the restrictions and requirements set by international health standards, the review focuses on plasma interaction with prokaryotic cells (bacteria), eukaryotic cells (mammalian cells), cell membranes, DNA etc. In so doing, some of the unfamiliar terminology—an unavoidable by-product of interdisciplinary research—is covered and explained. Plasma health care may provide a fast and efficient new path for effective hospital (and other public buildings) hygiene—helping to prevent and contain diseases that are continuously gaining ground as resistance of pathogens to antibiotics grows. The delivery of medically active 'substances' at the molecular or ionic level is another exciting topic of research through effects on cell walls (permeabilization), cell excitation (paracrine action) and the introduction of reactive species into cell cytoplasm. Electric fields, charging of surfaces, current flows etc can also affect tissue in a controlled way. The field is young and hopes are high. It is fitting to cover the beginnings in *New Journal of Physics*, since it is the physics (and non-equilibrium chemistry) of room temperature atmospheric pressure plasmas that have made this development of plasma health care possible.

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Contents

1. Introduction

Plasmas have been used for a long time for sterilization of medical equipment, packaging in the food industry, implants, blood coagulation, etc [1]–[8]. This is partly due to their high bactericidal effectiveness and partly due to their easy access into narrow and confined spaces [9]–[14]. In recent years, cold—less than 40 °C at the point of application—atmospheric plasma (CAP) sources have been developed that (in principle) provide the possibility to extend plasma treatment to living tissue. This opens up new horizons. Not only is there the vision of rapid, contact free sterilization, which can access even small pores and microscopic openings,

but also one may envisage new possibilities of drug delivery at the molecular level, new biomedical effects due to ions and, in the distant future, maybe even new plasma drug developments operating at the cellular level that may act selectively and/or regeneratively. Much of this will depend on the ability to 'design' plasmas chemically, to produce, transport and apply plasmas physically and to determine the plasma-tissue effects quantitatively using cell and microbiology, followed by medical studies.

Plasmas can be produced by various means, e.g. radio frequency, microwave frequencies, high voltage ac or dc, etc. During the non-quibilium processes both excited species and reactive gases may be produced (as well as ions and electrons) and it is these species that deserve particular attention for health care.

The field of, more generally, **plasma health care**, is an emerging field that has its roots, quite naturally, in plasma science. It has grown rapidly, however, and is now the subject of a broad interdisciplinary research effort involving medicine, biology, physics, chemistry and engineering. In this 'Focus Issue' of the New Journal of Physics we summarize contributions from the latest research activities across these disciplines. To introduce the topic and to familiarize the interested reader with some of the terminology used, we found it expedient to include this introductory review.

In the course of this 'Focus Issue' there will be publications on **plasma sources** and designs, statements about safety limits (always important for new medical devices and applications), non-equilibrium **chemistry** initiated by plasma interactions, **plasma diagnostics**, etc.

From **microbiology** there will be investigations with bacteria (gram-positive and gram-negative, which are common types with different cell wall structures), fungi, spores and viruses, which can all be destroyed using plasmas with varying degrees of effectiveness.

In **cell-biology**, the difference between 'prokaryotic cells' (bacteria) and 'eukaryotic cells' (e.g. skin fibroblasts or epithelial cells) will be discussed, as will the cell cycle with its different phases including DNA replication and cell division. Cell death (apoptosis), cell proliferation, arrest of cell cycle phases all play a role in the biological applications—why and how will be briefly discussed.

From the **medical** area there is an early report [15] on the use of Ar plasma in skin surgery. For chronic wound treatment with Ar plasma, so far only one clinical study has been started [16, 17] and the Phase II is near completion with over 1000 treatments carried out already. Air plasma devices have been also used in medical practice as a source of gaseous nitric oxide. The results of animal studies and clinical trials showed that NO-containing gas generated by plasma is effective in tissue disinfection and regulating inflammatory processes associated with acute and chronic wounds [18]–[20] and respiratory problems [21].

In particular, we point the reader to the following publications in this issue [22]–[32]:

- Removal and sterilization of the biofilms and planktonic bacteria by microwave-induced argon plasma at atmospheric pressure [22].
- Cell permeabilization using a non-thermal plasma [23].
- Generation and transport mechanisms of chemical species by a post-discharge flow for inactivation of bacteria [24].
- Designing plasmas for chronic wound disinfection [25].
- Low pressure plasma discharges for the sterilization and decontamination of surfaces [26].

- Inactivation factors of spore forming bacteria using low-pressure microwave plasmas in N_2 and O_2 gas mixture [27].
- Application of epifluorescence scanning for monitoring the efficacy of protein removal by RF gas-plasma decontamination [28].
- A novel plasma source for sterilization of living tissues [29].
- The effect of low-temperature plasma on bacteria observed by repeated AFM imaging [30].
- Physical and biological mechanisms of direct plasma interaction with living tissue [31].
- Nosocomial infections—a new approach towards preventive medicine using plasmas [32].
- Portable air plasma torch contributes to rapid blood coagulation as a method of preventing bleeding [33].
- Acidification of lipid film surfaces by non-thermal DBD at atmospheric pressure in air [34].
- Degradation of adhesion molecules of G361 melanoma cells by a nonthermal atmospheric pressure microplasma [35].
- Reduction and degradation of amyloid aggregates by a pulsed radio-frequency cold atmospheric plasma jet [36].
- A two-dimensional cold atmospheric plasma jet array for uniform treatment of large-area surfaces for plasma medicine [37].

Amongst envisaged applications are

- **Hospital hygiene**: Particularly, the growth of resistant bacteria (e.g. MRSA) poses a problem that requires fast and efficient sterilization [38]. Plasma devices that can do this are being developed and the expectation is that these will make a big difference. Of course, plasma (hand) sterilization is not restricted to hospitals—community associated infections, as opposed to nosocomial (hospital induced) ones are growing rapidly, too. Therefore all public buildings, children nurseries, nursing homes, etc would benefit from such a device.
- **Antifungal treatment**: It has been shown that plasmas can be employed efficiently to combat fungal diseases [32]. The plasma effect even propagates through socks. Common diseases such as tinea pedis (athlete's foot), which are believed to affect 25–40% of the population in Europe, the US and Japan, can therefore be treated quite effectively using appropriate plasma devices in the home or in medical practices.
- **Dental care**: 23% of over 65 year olds and over 75% of pregnant women suffer from periodontal infections [39]. These infections, in turn, increase the risk of heart diseases and other medical complications. Plasmas, with their ability to penetrate into microscopic openings between tooth and gum, seem ideal candidates for prophylactic treatment in addition to normal dental care.
- **Skin diseases**: Most dermatological problems are associated with bacterial or fungal (side) effects. There are over a thousand skin diseases ranging from acneiform eruptions, dermatitis, melanocytic (cancerous), pruritic to vascular related afflictions. Whilst plasmas are unlikely (based on our current technology) to cure such diseases, they can help to reduce complications through bacteria and fungi. In future, with 'designer' plasmas becoming a 'molecular drug delivery agent', even treatments of some of the diseases themselves may become possible.

- **Chronic wounds**: About 1% of the population in developed countries is suffering from chronic wounds [40]. There are different causes, e.g. venous diseases, arterial diseases, diabetes mellitus, pyoderma gangraenosum, carcinoma. As the population ages, the occurrence is likely to increase. Again, plasmas may help with the treatment—and again it is unlikely that plasmas will 'cure' the underlying disease. But by eliminating bacterial and fungal infections, plasmas may well reduce the suffering, support the treatment and speed up the recovery.
- **Cosmetics**: Plasma treatment for cosmetic re-structuring of tissue [41]–[44] has been discussed, as well as skin rejuvenation using nitrogen plasmas [45, 46]. Tooth bleaching is also enhanced by plasmas, mainly due to the *in situ* production of hydrogen peroxide [47].

There are other envisaged applications, ranging from blood cleansing, pharmaceutical processes to cancer treatment, but these are not developed sufficiently far at present to go into details—nevertheless they remain fascinating research topics. For further material on these and additional topics the reader is referred to three other reviews—by Laroussi [11], Fridmann *et al* [3] and Heinlin *et al* [48].

A final note for this introduction. The human body contains about 1.5 kg of bacteria—around 100 trillion microbes—with a few per cent residing on the largest organ, our skin [49, 50]. Most of these bacteria are not pathogens that induce diseases and possibly death—they are even beneficial to our well being (so-called 'commensal organizms'). Plasma treatment, like antibiotics, does not differentiate between 'good' and 'bad' bacteria yet, although with more research the treatment might be made more specific in future. With present technology, plasma treatment reduces the bacterial load in the treatment area—*in vitro* tests have resulted in reductions of up to a factor 1 million in a few seconds. Some bacteria invariably survive—as indeed they do in the case of antibiotics, too. Hence the population can—and will—regrow. Typically every 20 min the bacteria number doubles[6]. This implies the following.

If the bacterial load is 1 million cm^{-2} and plasma treatment has successfully reduced this number to 1 cm^{-2}, then it will typically take 10 h for the population to regrow to its former equilibrium value. This will be the case for the commensal organizms as well as for the pathogens.

The important difference is, however, that in the case of the pathogens the plasma treatment is providing the normal immune system with a better chance to combat the threat in its own natural way.

The above numbers illustrate that the (future) plasma treatment should be continued on a once/twice per day basis until the threat of infection has been overcome. This is basically similar to the usual antibiotic applications, the difference being the (at present) restriction of plasma treatment to the body surface, the almost instantaneous (few seconds) bactericidal response to the plasma application, the lack of a microbial defence system (and hence resistance build-up) to plasmas and the comparatively low level (or even absence) of unwanted side-effects, as far as we know, to suitably designed plasmas.

[6] The bacterial growth rate depends on the availability of nutrients and environmental conditions—see e.g. charts provided by the Alberta Health Services and others.

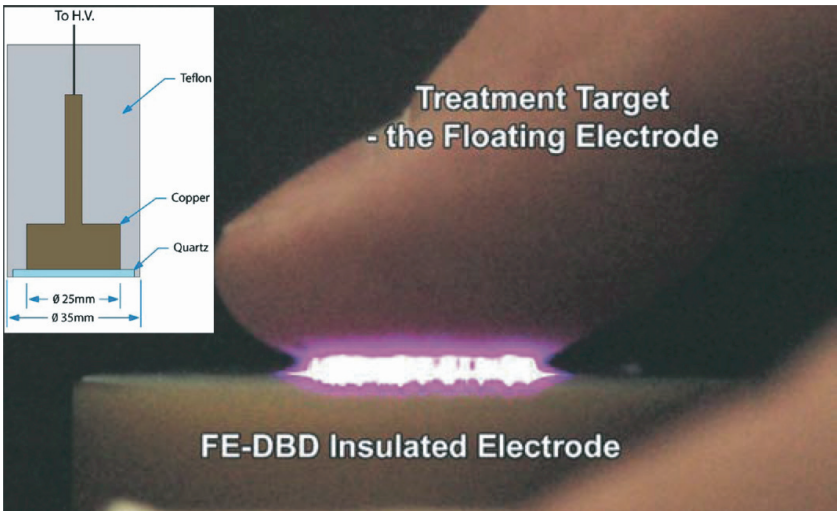

Figure 1. Plasma produced by the Floating-Electrode Dielectric Barrier Discharge (FE-DBD) electrode and schematic design of the FE-DBD electrode (top left) [52].

2. General requirements for plasma health care

We restrict ourselves for the remainder of this review to CAPs, since these are the most recent technological development with relevance to health care.

CAPs come in (basically) three types:

1. **Direct plasmas**, which use the skin (or other tissue) as an electrode so that the current produced has to pass through the body. The most widely used technology is the 'dielectric barrier' plasma source [3, 51, 52] (figure 1 [52]).
2. **Indirect plasmas**, which are produced between two electrodes and are then transported to the area of application entrained in a gas flow. Different devices exist, from very narrow 'plasma needles' to larger 'plasma torches' [11, 13, 47], [53]–[60] (figures 2–5 [53]–[56]).
3. **'Hybrid plasmas'**, which combine the production technique of (1) with the (essentially) current-free property of (2). These are also called 'barrier coronal discharges (BCD)'. This is achieved by introducing a grounded wire mesh electrode, which has much smaller electrical resistance than the skin—so that practically all the current passes through the wire mesh [32] (figure 6 [32]).

Plasmas can be produced by discharges in air, in noble gases or in any desired mixture in order to produce a 'chemical cocktail' of atoms, ions and molecules for biomedical applications. General requirements for all possible plasma components do not exist. Current safety constraints for all plasma sources refer to general electrical (e.g. VDE) safety regulations, to ultraviolet (UV) production, reactive species (RS) production and current limits through the skin.

2.1. UV radiation

UV light, especially at around 260 nm (UVC), initiates a reaction between two pyrimidine molecules (thymine and cytosine) adjacent to each other on the same strand of DNA causing

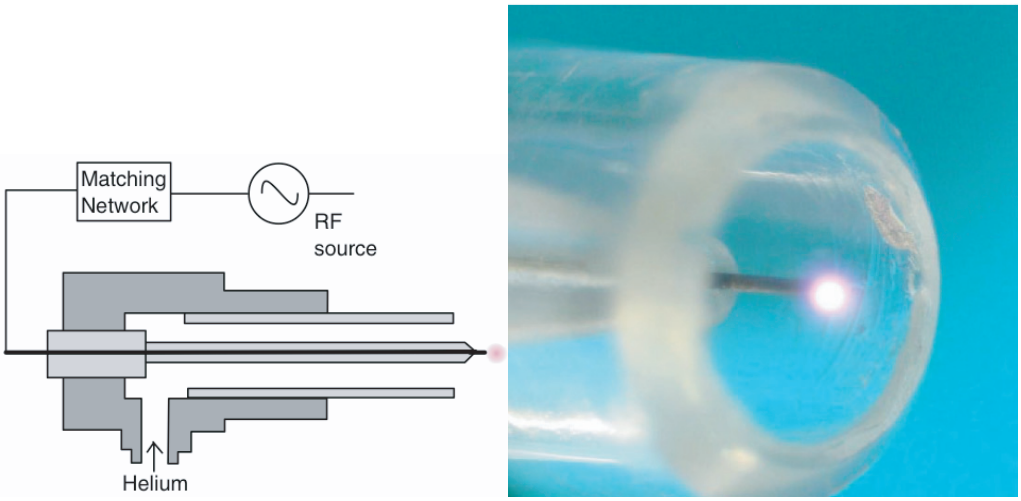

Figure 2. Schematic drawing of the plasma needle set-up (left) and plasma generated by the plasma needle (right) [53].

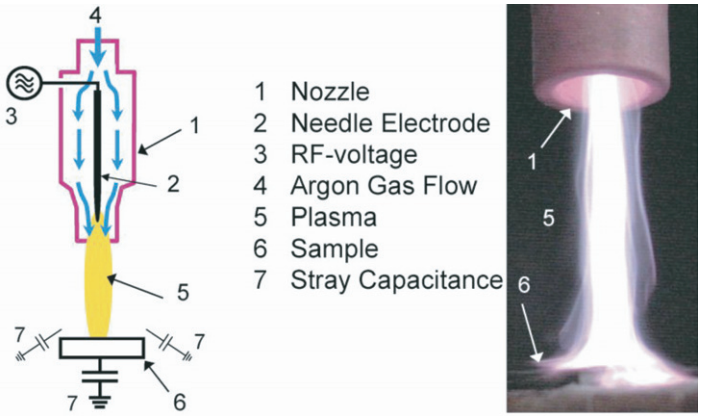

Figure 3. Atmospheric pressure plasma jet (APPJ) for the treatment of punctually inoculated test strips: schematic set-up and photo taken with 8 ms of exposure time [54].

them to form a dimer. The formation of pyrimidine dimers is the most important type of UVC damage. The presence of a pyrimidine dimer in the DNA affects base pairing and can cause mutations during DNA replication (synthesis of a complementary DNA strand). Exposure to high doses of UV radiation can cause mutagenesis and cell death. More details can be found in section 3. The 'safe' UV dose is estimated (for healthy skin, which is protected against UVC through a thin outer layer—the stratum corneum) by folding the UV production spectrum with an 'erythemal weighting function'. The maximum allowed dose rate is 30 μW cm^{-2}. (For more details see SCCP European Commission Report 0949/05.)

For plasma (or UV) treatment of unprotected cells (skin damage and wounds) no comparable regulations exist at present. Ethically, the (faint) possibility of mutagenous cell damage may be regarded as an acceptable risk when balanced against possible benefits in the treatment of other serious diseases, but this is a very difficult issue. The best approach is to design plasmas with as little UVC as possible.

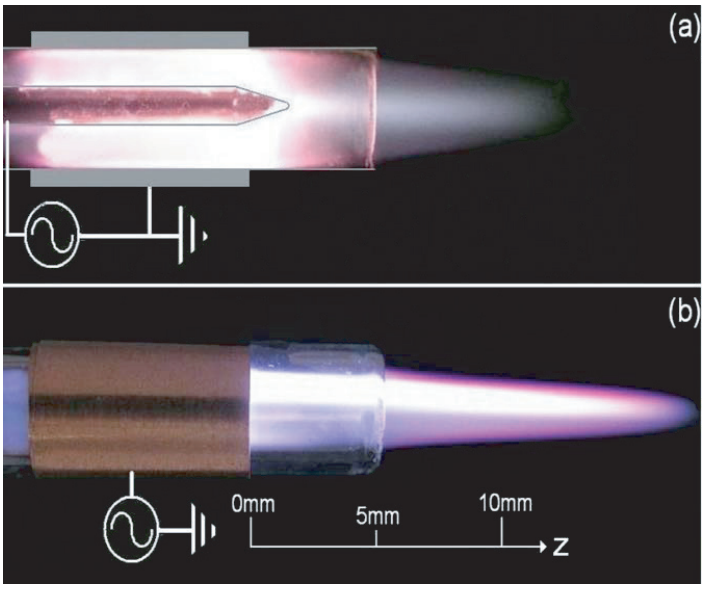

Figure 4. Image showing (a) a cross-field plasma jet and (b) a linear-field jet. Both jets were sustained at 15 W input rf power at 4 MHz [55].

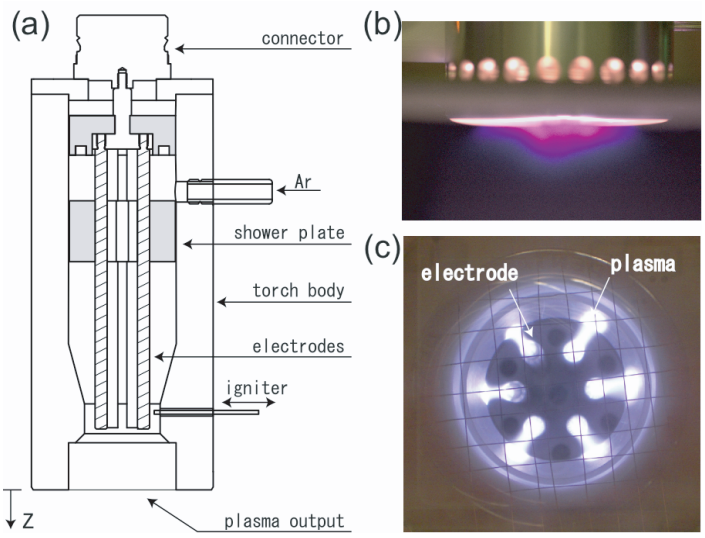

Figure 5. Schematic view of the plasma torch system and produced plasma: (a) plasma torch, (b) plasma below the torch and (c) plasma produced between the electrodes and the cylinder [56].

2.2. Reactive molecules

The major reactive molecules produced in atmospheric pressure plasmas are initiated by dissociation reactions of plasma electrons with atmospheric oxygen and nitrogen. The resulting chemical network involves over 200 important reactions, with products such as O_3, NO, NO_2, etc. The CPSC (Consumer Products Safety Commission) Report from 26/09/2006 recommends an upper Ozone limit of 50 ppb. More detailed studies by R Corsi point to a factor 10 lower

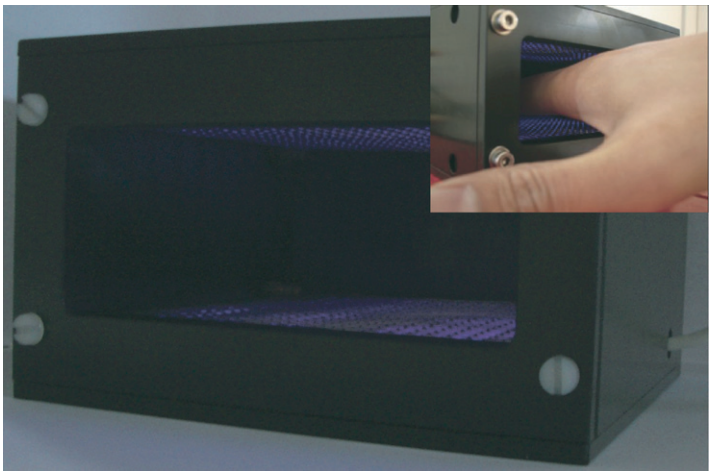

Figure 6. Plasma produced by the 'HandPlaSter' prototype, with 18 kV_{pp} at 12.5 kHz. The inset shows the application (hand disinfection) [32].

values. However, they have not been officially adopted so far. Regarding the nitrogen species, the US National Institute for Occupational Safety and Health (NIOH) suggests a permitted exposure limit of 5 ppm for NO_2 and 25 ppm for NO over an 8 h period. Several countries have regulated the NO_2 threshold at 2 ppm.

2.3. Electrical currents

Since plasmas consist of electrons and ions, charges may flow—i.e. currents may pass through the skin in medical applications. The natural resistance of dry skin is 10 kΩ. In the presence of a grounded conductor this implies that virtually no current will pass through the skin. This is the case for the 'hybrid' BCD plasmas. However, in the traditional DBD devices, the skin is an electrode by design. Of course the currents are small—typically a few mA to a few tens of mA. The ICNIRP current limits are 0.5 mA at 1 kHz, rising to 20 mA at 100 kHz. These levels were based on 'touch perception' by test persons (and relate mainly to 'creepage' currents in electrical devices). Since most DBD devices are operating at high frequencies, the ICNIRP current limits are not exceeded. Regarding the plasma 'jet' sources or torches, again currents through the skin can be neglected, although a small fraction of the discharge may pass through the skin. Suitable electrode designs have been developed that minimize this effect.

2.4. Required plasma doses

The 'dose'—or the amount of time over which the plasma is applied—depends on the plasma composition and the purpose. For bacterial sterilization, *in vitro* experiments have shown that a few seconds to a few minutes are necessary [11, 13, 32, 47, 56, 57]; for a recent review see [3]. Important in this context are the levels of 'unwanted' by-products, e.g. the integrated UV dose (which should be kept small for *in vivo* application) and the total level of RS has to remain below the WHO limits (although they are essential for the bactericidal efficiency of the plasma—so we have some opposing requirements). This may require particular designs, e.g. where the RS are filtered away after they have completed their sterilizing effects, or the use of catalysts as part

of the electrode designs. Plasma doses also can be adjusted to e.g. combine bactericidal effects on the one hand and tissue regeneration on the other [25]. This requires careful and extensive experimental tests.

2.5. Scaling of plasma devices

The different applications mentioned above (which for general health care may be extended to include air and water purification, pollution control, micro-filtering of aerosols, de-odorizing, etc) require different geometries, shapes and sizes for possible plasma devices. Consequently, a large number of designs has been developed and tested already. The ability to devise one specific plasma production design that can be scaled (and shaped) for a multitude of applications—and which works quickly, is an ongoing engineering challenge.

'Jet' sources have the advantage that the surface to be treated may be 'rough' on scales of micro- to millimetre, the disadvantage is the limited size of the jet (mm), which requires mechanical translation or multiple jet designs [61]. Plasma torches [56] have much larger areas, about 25 cm^2 with a power consumption of about 100 W. They are suitable for larger area treatments. 'DBD' sources have the advantage that they can be produced (in principle) to cover even larger areas and they can be shaped—however, the surface has to be smooth enough ($\ll$ mm), otherwise the discharge becomes inhomogeneous and treatment is 'patchy'. The 'Hybrid' BCD sources combine the positive aspects of size and shape with the independence of surface structure. They are a fairly recent development, with sizes of 200 cm^2 tested so far at a power consumption of 0.5 W cm^{-2}. Their competitiveness with the more established (DBD, jet torch) technologies still needs to be assessed, but they appear to be quite promising.

2.6. Biomedical plasma simulation studies

In the past five years, a number of computational studies have been dedicated to microplasmas of interest to biomedical applications.

In 2005, Brok *et al* [62] used the Plasimo/MD2D model to simulate the plasma needle in helium with a small admixture of nitrogen. This study was based on a fluid model that encompassed a self-consistent description of the electrostatic field, drift-diffusive motion of the plasma species and a calculation of the mean electron energy. By solving the electron energy balance, rather than relying on the local field approximation (LFA), non-local electron heating could be accounted for. For an excellent comparison of the electron energy balance, the LFA and more rigorous methods like Particle-in-Cell/Monte-Carlo methods, we refer to [63]. The study of Brok *et al* revealed that even for nitrogen abundances down to 0.1%, N_2^+ is the dominant ion species. The study did not take the possible heating of the background gas into consideration.

Starting in 2006, Sakiyama *et al* followed up on this work with a series of publications regarding the plasma needle. In [64], they presented simulation results that were based on finite-element calculations. This allowed the authors to obtain a much better spatial resolution in the region near the powered electrode than that which Brok *et al* had been able to achieve. On the other hand, this work assumed the LFA, rather than solving the mean electron energy equation as Brok *et al* did, which is a simplification of Brok's physical model.

In [65] further investigations on the plasma needle were made and it was possible to demonstrate two modes of operation. For low plasma powers, the plasma was shown to operate in a corona mode, in which Penning ionization is the dominant reaction forming N_2^+, and large variations of the charged species densities can be observed during an RF-cycle. Above a certain

power threshold, the plasma makes a transition to a glow mode, which is dominated by (direct) electron impact ionization of helium. The authors favourably compared their simulation of the mode transition with experimental studies that previously indicated that such transition may occur.

In [66], a detailed analysis of the structure of the sheath was made using an atmospheric RF argon plasma with a small admixture of nitrogen. This model assumed a simplified one-dimensional spherical geometry, but this time the mean electron energy was calculated from the electron energy equation, rather than assuming the LFA. In addition, in this paper, the authors calculate the gas temperature from the heat balance equation. The authors also discuss the fact that the LFA did not yield converging results in the simulations of discharges at higher powers.

The influence of the electrical properties of the target surface were investigated in [67]. There it was predicted that for a conducting surface, the flux densities are strongly peaked at the closest surface point. On the other hand, for dielectric materials and low plasma powers, the flux density resembles a Gaussian profile and is primarily due to the remote production of plasma species. For higher powers, when the plasma makes the transition to the glow mode, a relatively uniform ionization layer is formed in a plasma sheath close to the target surface; this results in more uniform flux densities.

In [68], a detailed discussion of the influence of the gas flow on the discharge structure was provided. In this paper, it is suggested that, above all, the gas flow hampers the entrainment of the ambient N_2 gas into the discharge region. As a result, the ionization of N_2 by excited helium molecules that are produced in the discharge region happens mostly in an off-axis annular region. These predictions are in qualitative agreement with the results of experiments carried out by Goree *et al* [69].

The above-mentioned paper is the most complete numerical description of a biomedical plasma source available at present: it self-consistently takes into account the flow and heating of the buffer gas, the electrostatic potential and the plasma composition. Yet it is far from feature complete. In particular, the model assumes that the plasma is operated in a pure nitrogen environment, rather than air, so oxygen-containing species are not taken into account. Furthermore, the plasma composition may be affected by turbulent mixing [69], which is not dealt with in Sakiyama's paper [68]. In addition, the validity of the fluid approach must be analysed in greater detail [63].

All computational studies we have mentioned so far concerned the plasma needle. A lot of research has been done on other atmospheric glow discharges with potential biomedical applications, albeit in different contexts. As an example, micro discharges have been extensively studied in the context of Plasma Display Panel (PDP) technology (see for example [70] and the references therein). Other numerical microcell studies with a high level of sophistication are discussed in [71, 72] (as well as the references therein).

It seems that the models that underlie these studies can be modified in order to simulate sources of biomedical interest. The key challenge is to deal with plasmas in more complex gas mixtures, like humid air. The much more complex chemistry is a challenge on its own, but introduces an indirect complication as well: the presence of multiple timescales. The (primary) electronic processes in the plasma (like direct impact excitation and ionization) happen on the micro-second timescale, while a (periodic) steady-state solution may not be obtained before the chemical process involving heavy particles have settled, which may take milli-seconds or even seconds, depending on the system details. In addition, afterglow effects introduce yet another timescale (e.g. due to recombination).

Summarizing, there is a high level of innovation in designing CAP devices for bio-medical applications. The health care needed for new approaches to some of the most pressing (global) problems (e.g. MRSA) is great. Plasmas may play an important role in this field.

3. Biologically active plasma-generated agents and their application in medicine

The mechanisms by which low-temperature atmospheric pressure plasmas affect viruses, bacteria and eukaryotic cells are based on the synergy of several biologically active plasma components. The most important of them are plasma-generated UV radiation and RS including free radicals and some ground state molecules such as peroxides and ozone. Under certain conditions, heat, charged particles and metastable-state molecules and atoms produced by plasmas may also play important roles in the interaction between plasma and biological systems.

3.1. Effects of UV radiation

The effects of UV radiation on biological systems have been intensively studied at the molecular, cellular and organizmal levels. These effects show a strong dependence on the UV dose and wavelength. Thus, the small amount of the solar UVB (290–320 nm) radiation that reaches the Earth surface is a prerequisite for the vitamin D synthesis in the human skin [73]. Solar UVA radiation (320–400 nm) controls the biosynthesis of hormones involved in the regulation of the diurnal or circadian rhythmicity in mammals (including humans) [74]. However, a long-term exposure to UV radiation is known to cause skin erythema, keratitis and ultimately may lead to skin photoageing, cancer and cataract [75].

There are two main mechanisms for the UV-induced cellular damage at the molecular level: (i) direct effects of UV radiation that are based on the UV energy absorption by cellular macromolecules and (ii) DNA, protein and lipid alterations caused by the UV-induced disturbance in the cellular redox state (oxidative stress). Direct effects of UV radiation include modifications of DNA, such as cyclobutane pyrimidine dimers and pyrimidine(6–4) pyrimidone photoproducts [76, 77] and conformational rearrangement and aggregation of the protein polypeptide chains [78]. Besides the direct modification of macromolecules, UV radiation induces the release of intracellular RS [79, 80], which in turn cause oxidative degradation of lipids (lipid peroxidation) [81] and oxidative DNA damage [82]. The high-energy short-wavelength UV (UVC; 200–290 nm) is the most damaging type of UV radiation. Sterilization with germicidal lamps relies mostly on the effect of UVC on bacterial DNA and proteins [83]. In eukaryotic cells, a significant fraction of UV-induced DNA damage is repaired by the nucleotide excision repair system [84]. However, the DNA repair system makes errors. Chronic exposure to subtoxic doses of UV radiation leads to the accumulation of these errors (mutations) and resulting genetic instability of cells [82]. Similar effects can be achieved by cell exposure to high concentrations of exogenous RS such as superoxide radicals, hydrogen peroxide, singlet oxygen and hydroxyl radicals.

3.2. RS

RS have been traditionally regarded as hazardous by-products of cellular metabolism and are implicated in the development of a wide array of human diseases [85]. However, it has been known for a long time that RS produced by mammalian immune system cells, macrophages

and neutrophils, play a fundamental role in the antibacterial and antiviral defence [86, 87]. Accumulating evidence suggests that RS are also involved in the regulation of cellular functions. In multicellular organizms, regulation of diverse physiological processes relies on a complex network of intracellular and extracellular signals. Typically, extracellular signal molecules (hormones, growth factors, cytokines and neurotransmitters) bind to specific cell surface receptors and by virtue of this activate intracellular signal cascades that ultimately lead to the activation of transcription factors involved in the regulation of gene expression. RS appear to be involved in the regulation of a large number of signalling pathways at multiple levels from receptors to nucleus (reviewed in [88]). It has been shown that exogenous RS may activate upstream proteins in signalling pathways such as growth factor and cytokine receptors [89, 90] as well as downstream transcription factors [91, 87]. The two general mechanisms of the RS signalling are: (i) alterations in intracellular redox state and (ii) oxidative modification of proteins involved in signalling pathways [88].

Being signal molecules, RS are involved in essentially all physiological processes in the human organizm including regulation of vascular contraction, blood coagulation, angiogenesis, inflammation, immune system response and nerve impulse transmission [93]–[96]. At the cellular level, RS regulate cell differentiation, division, migration and apoptosis [97]–[100]; they control cell-to-cell adhesion [101], biosynthesis of growth factors and collagen production [102, 103]. Cellular responses to different types of RS depend on their concentration. For instance, depending on concentration, hydrogen peroxide (H_2O_2) either stimulates or inhibits cell proliferation or induces cell apoptosis [88]. Nitric oxide ($NO^{\cdot}$) at low concentration acts as anti-inflammatory agent that inhibits lipid peroxidation and protein oxidation by reactive oxygen species, diminishes membrane permeability and limits cell apoptosis [97]–[108]. However, when it is formed at high rates, $NO^{\cdot}$ contributes to cell and tissue injury [109]–[110].

The interplay between diverse RS adds to the complexity of cell regulation. Regulation of blood coagulation by superoxide (O_2^{-}) and $NO^{\cdot}$ is probably the best-studied example of such an interplay [96, 112]. $NO^{\cdot}$, which is known as an anticoagulative factor, prevents platelet aggregation under physiological conditions. O_2^{-} released by activated or dysfunctional endothelial cells indirectly affects platelet activity by scavenging $NO^{\cdot}$ and by virtue of this induces blood coagulation. Another remarkable example of the RS synergy is the regulation of cell apoptosis by $NO^{\cdot}$ and H_2O_2. In a concentration-dependent manner, $NO^{\cdot}$ can either inhibit or enhance the hydrogen peroxide-mediated apoptosis in different cell types [111], [113]–[116]. Interestingly, the toxic effect of H_2O_2 on bacteria is induced even by low-concentration $NO^{\cdot}$. It was proposed that one of the mechanisms of the antimicrobial macrophage defence relies on different effects of $NO^{\cdot}$ and H_2O_2 synergy [118] (see [25] for more detail).

The physiological potential of UV radiation and RS has been used for therapeutic purposes on a regular basis. UVB and UVA radiation phototherapy is an approved method of clinical treatment of psoriasis, eczema, polymorphic light eruption, vitiligo and of many other inflammatory dermatoses [118]–[120]. $NO^{\cdot}$ and $NO^{\cdot}/O_2$ inhalation therapy is used to treat primary pulmonary hypertension and pulmonary vascular disease in infants [121]. Chemical compounds that release $NO^{\cdot}$ under physiological conditions, are widely prescribed in the case of hypertension, angina and heart failure (e.g. nitroglycerin, amyl nitrite and S-nitrosothiols) [122]. RS and their chemical carriers are also used for topical medical applications. Thus, high-concentration (3%) H_2O_2 solution is used clinically for wound disinfection. Recent studies show that topical application of low (0.15%) concentrations of H_2O_2 stimulates the wound closure process by activating redox-dependent pathways [123]. Gels and polymers releasing $NO^{\cdot}$ were

shown to be effective for the treatment of diabetic foot ulcers [124]. It has been proposed that the RS-donating compounds should replace growth factors in chronic wound therapy [125]. An uncontrolled kinetic of RS release is the major problem for using such compounds for topical applications. The development of plasma devices generating pharmacologically appropriate doses of RS would resolve this problem and open a new window of opportunity in the novel medical treatment methods.

4. Plasma interaction with prokaryotic cells

4.1. Overview

Although ionized gases have been known to be biocidal for centuries, the first report of inactivation data using CAPs was published only 14 years ago in 1996 [126]. Since then, the main interest in CAP interaction with prokaryotic cells has been the provision of a hitherto unavailable low-temperature solution to medical decontamination, particularly sterilization of surgical instruments and medical devices [6]. CAP decontamination of inanimate objects represents a major component of plasma medicine with the most numerous number of active research groups, the most comprehensive data of efficacy, and indeed the furthest advance towards widespread practical use so far. This is the first arena of plasma interaction with prokaryotic cells. With no direct exposure of human to gas discharges, there is relatively little toxicology concerns such as long-term impact on living human tissues by plasma-generated UV photons, RS and negative ions. However, CAP-based medical sterilization has its unique challenge and this is the inactivation of prions, a misfolded protein that defies any current commercial decontamination procedures [127]. Responsible for transmissible spongiform encephalopathy (TSE), in particular Creutzfeldt–Jakob disease (CJD) in human and bovine spongiform encephalopathy (BSE) in cattle, prions are far more resistant to external stresses than bacteria, virus and fungi. Prion decontamination represents the ultimate challenge in medical sterilization and more generally in decontamination of inanimate surfaces.

In addition to decontamination of inanimate surfaces, the generic bactericidal capability of CAPs has led to their use for decontaminating biological surfaces such as plant, animal and human tissues [1, 3, 5, 128, 129]. This represents the second arena of CAP interaction with prokaryotic cells, and is motivated by a number of healthcare needs, for example food decontamination, skin diseases and disinfection of living tissues in open wound. In such scenarios, plasma interaction with plant or human tissues is inevitable thus posing toxicology questions, for example possible plasma damages of healthy tissues [3] and possible compromised inactivation efficacy when bacteria migrate away from tissue surface into the bulk of the tissue [129]. Therefore the delivery of bactericidal plasma species must be appropriately weighed and timed, highlighting the critical need to understand the identities of key plasma species and their critical concentrations that induce bacterial inactivation with little toxicology consequence. There are therefore at least two arenas of research into plasma interaction with prokaryotic cells, namely decontamination of inanimate and of biological surfaces.

4.2. Bacterial and biomolecule inactivation

CAPs have been shown, by many different groups, to be very effective against gram-negative bacteria, gram-positive bacteria, spores, biofilm-forming bacteria, virus and fungi. Broadly

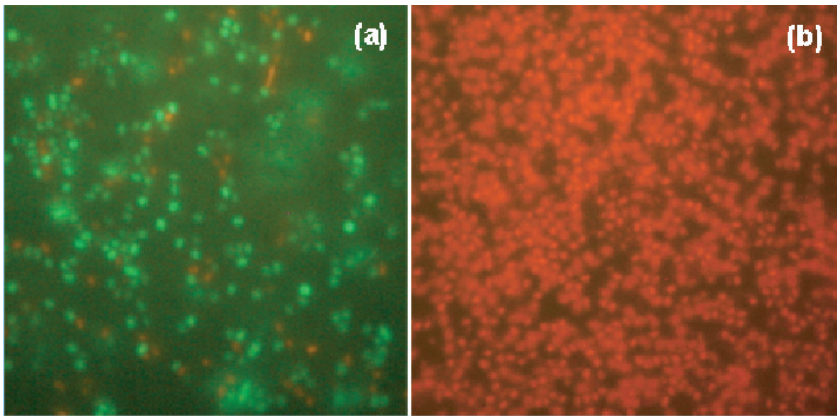

Figure 7. *B. subtilis* spores (a) before and (b) after CAP treatment. Green and red florescence indicate intact and bleached membranes [130].

speaking, the generic capability of CAPs against microorganizms is well established under controlled laboratory conditions [11]. Much has been learnt about some specific features of their bactericidal effects. For example, CAPs are far more effective against gram-negative than gram-positive bacteria and they can cause severe damages to membranes of microorganizms as shown in figure 7, where the *Bac*Light method is used to identify intact and bleached membranes of *B. subtilis* spores with green and red florescence, respectively [130]. The plasma used in the treatment was a room-temperature cold atmospheric He–O_2 plasma jet excited at 30 kHz. Similar results have also been observed in vegetative cells of bacteria. Key membrane components include lipopolysaccharide (LPS), phospholipid, peptidoglycan and membrane proteins. It is possible that CAPs cause a limited number of punctures through the membrane and induce irreversible damages to some membrane components, leading to cell death without altering the physical appearance of the microorganizm. This may be related to a sequence of cascaded events, both physical and biochemical, triggered by CAP impact and eventually the gradual death of a microorganizm. Equally it is possible that CAPs deliver a much more severe damage to the physical structure of the microorganizm. Figure 8 shows SEM images of the same *B. subtilis* spores before and after treatment by the same CAP He–O_2 jet [130]. It is evident that the impact of the CAP jet ranges from severe rupture (marked with an arrow) to little physical alteration (unmarked). This suggests at least two types of CAP impact, firstly a severe and blanket damage to the physical structure of a microorganizm that is likely to cause rapid cell death, and secondly less severe punctures that may trigger an irreversible sequence of physical and biochemical changes leading to a more gradual cell death.

Some bacteria produce biofilm, an extracellular polysaccharide matrix in which bacteria are embedded. Biofilms may form on living or non-living surfaces, and represent a prevalent mode of microbial life in natural, industrial and hospital settings [131]. Examples include *Pantoa agglomerans* and *Streptococcus mutans*, which are important in food processing and dentistry, respectively, and can be inactivated by CAPs [132]–[134]. However, a biofilm forms a physical shield and restricts direct exposure of bacteria to plasma species, thus leading to compromised inactivation [132]. It is possible that plasma agents such as RS, UV photons and charged particles can be used to cause damage to the biofilm itself. This is an area of current

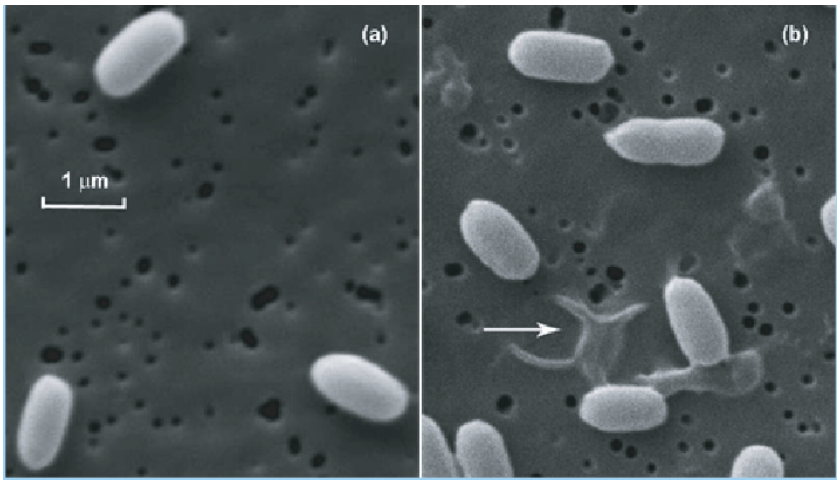

Figure 8. SEM images of spores of *B. subtilis* (a) before and (b) after treatment of a He–O_2 CAP jet. The arrow in (b) indicates ruptured spores [130].

interest for example in the case of plasma oral hygiene and root canal cleaning [47, 135]. The issue of physical shielding is also important when CAPs are used to treat surface-borne cell populations in which cells are easily stacked in many thousands of layers [136, 137]. When such stacked cell populations are treated by CAPs, cells in the uppermost layers are inactivated first. However, the inactivated cells remain in the uppermost layers and as a result they become a physical shield for cells located beneath the uppermost layers. Therefore in a laboratory study, it is important to distinguish the susceptibility of individual cells to plasmas from that of a cell population.

Many microorganizms that have been shown to be vulnerable to CAP treatment are directly relevant to some of the most important healthcare challenges. One example is hospital acquired infections, also known as nosocomial infection, caused by mainly MRSA and *Clostridium difficile* among others [138, 139]. The former is gram-negative, whereas the latter is gram-positive. One major cause of hospital acquired infection is human contact with a hospital environment of poor hygiene, which is at present addressed often by diligent applications of washing, detergent and hydrogen peroxide vapours. The persistence of hospital acquired infection in many different countries highlights the inadequacy of the current strategies and the need for alternative technologies. Figure 9 shows MRSA after treatment by an argon microwave atmospheric plasma torch. The microwave plasma device shown in the insert was developed jointly by the Max-Planck Institute for extraterrestrial Physics and Adtec Plasma Technology Co. Ltd. MRSA were plasma treated for 2 min and then incubated for 24 h. Where the plasma was applied, practically no surviving bacteria (identifiable as single colony forming units) are detected, indicating a bacterial load reduction of 99.9999%. These results suggest that CAPs are certainly capable of deactivating key microbial contaminants for hospital acquired infection. To advance these promising results towards a practical solution, two important challenges are (i) to scale up intrinsically small atmospheric plasmas for the physical environment of hospitals (e.g. floors, walls, beds and infrastructures) and (ii) to minimize environmentally unfriendly by-products (e.g. NO_x and ozone) so that CAP-based techniques can be applied in the proximity of patients. These demand pushing further the frontier of the current understanding of cold atmospheric physics and chemistry.

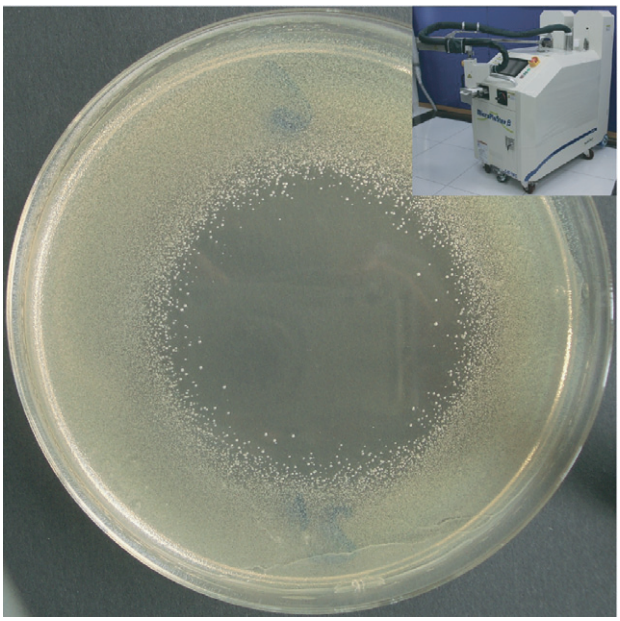

Figure 9. MRSA culture treated for 2 min with the MicroPlaSter device (insert upper right). A clear zone of inhibition is observed with an ∼5.3 cm diameter. (The distance between the opening of the plasma torch and the culture is 2 cm. Heat is not responsible for the bactericidal effect, since the gas temperature is ∼30 °C.)

Another major cause of hospital acquired infection is the reuse of inadequately sterilized surgical instruments, partly due to the difficulty to decontaminate small 3D or/and hollow features of some instruments and partly due to prion contaminants that render all commercial sterilization procedures useless. The second challenge highlights a relatively overlooked area within the plasma physics community that contamination of surgical instruments is not limited by microbial effects, but includes those by biomolecules such as proteins and human tissues [140]. Although it is yet to be fully established that non-equilibrium plasmas can significantly reduce prion infectivity, their capability to reduce protein models of prion has been reported including purified protein such as bovine serum albumin (BSA) and surgically acquired proteins [6, 141]. Figure 7 shows the reduction of BSA by a kHz CAP jet in helium–oxygen flow with the BSA labelled by a laser-induced florescence technique. The ultimate test for non-equilibrium plasmas including CAP would be against CJD agents in an appropriate animal model, the topic of which is itself in hot debate and highlights the complexity of prion diseases [142]. An equally essential challenge is whether CAP could be developed to treat effectively large and often three-dimensionally structured instruments as well as long lumens and catheters of diameters below 1 mm. This is not an engineering issue alone, since the need to deliver abundant reactive plasmas species over large areas and into narrow channels imposes many fundamental questions on stability of inherently small atmospheric plasmas when generated in electronegative gases.

It is important to revisit the ability of CAPs against microorganizms and proteins in a broader context beyond medical sterilization. Antimicrobial effects of CAP find applications in fungal diseases, skin diseases, root canal cleaning, pharmaceutical process control and food decontamination, to name just a few examples. The expansion of this basic CAP capability is

easily conceivable for other applications where microbial contamination is an issue, including recontamination of open wounds [3]. The ability of CAP to inactivate and degrade components of prokaryotic cells such as proteins has implications well beyond prion disease. For example, it has recently been recognized that CAP could inactivate adhesion proteins of cancer cells [143] thus offering a new route to plasma-assisted cancer therapy. Although these are early evidences in a long journey towards a successful therapeutic solution, the study of CAP interaction with cell components represents a more detailed enquiry at a cellular level (e.g. plasma biology) and may lead to new phenomena and new application implications (e.g. plasma medicine) not easily imaginable with today's understanding of plasma-cell interaction.

4.3. Inactivation mechanisms

Broadly speaking and in the context of plasma inactivation, mechanisms of CAPs with prokaryotic cells involve at least two aspects, namely cellular components and processes through which bactericidal plasma species inactivate (biological mechanisms) and bactericidal species delivered by CAP to cells (physical mechanisms) [11, 130]. The former is less understood, partly reflecting the fact that there have been few biologist-led investigations. However, different mechanisms have been proposed from the plasma physics community with different levels of supporting evidence. For example, membrane breaching has been substantiated (see figure 7 and [49]) and DNA damage has been perceived as less important in oxygen-containing CAP systems because of atmospheric absorption plasmas. In the case of membrane breaching, this could be caused by either physical punctures or oxidation of membrane proteins. It has also been suggested that the metabolism of prokaryotic cells can be significantly affected by CAPs [144] and this can lead to cell death. Many questions remain, including possible damage and degradation of CAP to other cell components such as LPs (in the case of gram-negative cells) and peptidoglycan (in the case of gram-positive cells), and possible cascading events in which one or few plasma-induced effects trigger an irreversible cell damage.

Comparatively less uncertain are possible physical mechanisms [3, 130, 145]. However, they depend on the chemical composition of the working gas mixtures as well as the temporal and spatial details of plasma generation. In oxygen containing CAP, particularly at excitation frequencies below the very high frequency (VHF) band of 30–300 MHz, reactive oxygen species are abundant including the ground and excited states of oxygen atoms, ozone and OH radicals (originating from moisture in the ambient air) [130, 144, 146]. In principle, oxidation potentials of ozone, ground state oxygen atoms and OH radicals are 2.07, 2.4 and 2.8 V, respectively. This would suggest a dominating role of OH radicals, however, the oxidation of prokaryotic cells also depends on the concentrations of the different species relative to their respective threshold concentrations beyond which cell damage is irreversible. In other words, the bactericidal effect of a particular plasma species depends on both its reactivity and its concentration, e.g. its plasma dosage. The efficiency measure is then possibly a weighted product of the two properties. The current understanding of CAP interaction with prokaryotic cells acknowledges the importance of plasma dosage as a concept but is primitive in terms of its quantitative measure. The latter is related to the difficulty to single out one particular plasma species in CAPs (in fact any low-temperature plasmas). Such plasmas tend to produce a cocktail of most RS with little opportunity to enable their individual production alone. In an attempt to shed light on which species of oxygen-containing CAP are definitely important and which are definitely minor players, a recent study employed bacterial mutants with specific repairing mechanisms

removed [147]. The study found that OH radicals and UV photons play minor roles in a typical helium–oxygen CAP, whereas oxidation is dominant. In this study, oxygen was about 0.5% of the background helium. Under such conditions, combination of two-photon laser-induced fluorescence experiments [148] and fluid simulation [149] suggest that the concentration of ground state oxygen atoms is about one order of magnitude higher than that of ozone. Given the difference in their oxidation potential, it is likely that oxygen atoms play a very important, if not dominant, role in bacterial inactivation of oxygen-containing CAPs [145]–[147].

It should be noted, however, that a significant change to the temporal character of plasma excitation could lead to the generation of more energetic electrons, which can in turn increase the production of UV photons. This could be done by using very short high-voltage pulses that have been shown to shift the electron energy distribution function (EEDF) towards higher energies and produce more UV photons [150]. In such cases, UV production may be raised towards, or even above, the critical dosage level, thus elevating the UV role in bacterial inactivation. Greater UV production is possible by increasing the excitation frequency well above the high frequency (HF) band of 3–30 MHz. There is some evidence that this may increase UV production at microwave frequencies [56], though studies of HF and VHF CAPs suggest that electron heating could be compromised by electron acceleration being 'starved' due to the short half period at high frequencies [151, 152]. Alternatively, UV production in nitrogen-containing plasmas is greater and this may be an interesting venue to pursue. In general, it is of great interest to pursue oxygen-based reaction chemistry for two reasons, firstly the ability of gaseous oxygen species to diffuse into small crevices in the context of surgical instrument sterilization and secondly the ability to control UV damage to healthy tissues in the context of wound healing. It is along this line of consideration that we place considerable emphasis on OH-based reaction chemistry, facilitated for example by CAP in touch with liquid or wet objects [153].

Charged particles and electric field can also play an important role, as proposed by Laroussi [14] and more recently discussed in greater detail [154]. The difficulty to measure charge densities and electric field makes it difficult to go much further than hypotheses, though recent success in measurement of electric fields in atmospheric microplasmas offers hope for greater support from the plasma diagnostics community. In general, the current studies of plasma–cell interaction mechanisms face the persistent difficulty of reliable measurement of electron density and electron energy, ion density and energy, and concentrations of radicals and negative ions for atmospheric plasmas. While an empirical strategy aimed at achieving an acceptable level of application efficacy may yield some dividends, it would be naïve to assume that this would be adequate given the complexity of plasma physics and chemistry of CAPs and the fact that new CAPs are being rapidly conceived with a similar expanse of unknown phenomena. Such complexity is compounded by the complexity in cellular responses in a multi-species cell population and in contact with human tissue. To provide a true plasma-assisted solution to healthcare, fundamental studies in plasma biology and plasma physics must go hand in hand with applied investigations of plasma medicine and plasma-assisted healthcare.

Our review of plasma interaction with prokaryotic cells has focused on the bactericidal effects of CAPs, and this shares some similarity with that of low-pressure gas discharges [10]. However, the interest in plasma interaction with prokaryotic cells goes well beyond biological decontamination. Indeed recent studies have ventured into modification of cell functions, particularly metabolism, so that plasma-treated prokaryotic cells could be encouraged to digest pollutants, increase hydrogen production and convert crude oil. Therefore plasma interaction

with prokaryotic cells has at least three arenas of research activities, sterilization of inanimate objects, disinfection of plant and human tissues and modification of cellular functions [155].

5. Plasma interaction with eukaryotic cells

In the following section, the emerging field of plasma medicine is discussed, where low-temperature atmospheric-pressure plasmas have a broad range of applications, including the disinfection of living tissues [52], blood coagulation [156], induction of apoptosis in malignant tissues [157], a localized modulation of cell adhesion and proliferation [158]–[163] and tissue modification of interest in electrosurgery [164].

5.1. Blood coagulation

Quasi-thermal plasmas in the form of the so-called cauterization devices have been used for a long time in medical practice for blood coagulation in wound treatment and surgery. The mechanism of blood coagulation by high-temperature plasmas is based on heat-driven tissue protein denaturation and blood desiccation. However, results of recent studies by Fridman *et al* [52] and Kalghatgi *et al* [165] indicate that high temperature is not a prerequisite for the plasma-induced blood coagulation. The authors show that plasmas can trigger natural, rather than thermally induced, blood coagulation processes. They performed *in vitro* experiments in which they exposed blood samples to low-temperature plasmas generated by the FE-DBD device. The rates of blood coagulation in plasma-treated samples were 15 times higher than in non-treated control samples. Studies using an animal model (SKH1 mice) demonstrated that low-temperature atmospheric-pressure plasmas enhance blood coagulation *in vivo* as well [141]. To specify the mechanism responsible for the plasma-assisted blood coagulation, authors performed additional experiments, which allowed the exclusion of factors such as the electric field, light, temperature, plasma-induced change of blood pH and the increase in the concentration of calcium ions due to their release from calcium-bound proteins [150]. They found that the plasma treatment activates the coagulation cascade by inducing the aggregation of the coagulation protein fibrinogen into fibrin. In turn, fibrin catalyses blood coagulation factors; this ultimately leads to the clot formation. The fact that the albumin structure was not altered by the plasma treatment suggests that plasma agents selectively trigger the polymerization of coagulation proteins. Low-temperature plasmas do not cause any pain or unpleasant smells usually associated with the thermal tissue treatment. Therefore it can potentially replace the quasi-thermal plasmas traditionally used in surgery.

5.2. Effects of low-temperature plasma treatment on mammalian cells

Studies on the effects of low-temperature atmospheric-pressure plasmas on mammalian cells have been conducted by several research groups [154, 156], [158]–[163]. Two major types of plasmas used in this research are the low-temperature atmospheric-pressure helium plasmas generated by plasma jet or needle devices and air plasmas produced by the dielectric barrier discharge (DBD) device. *In vitro* experiments with fibroblasts, endothelial and smooth muscle cells demonstrated that plasma affects cells in a dosage-dependent manner. Disruption of cell-to-cell adhesion and cell detachment from substrates were observed after cell exposure to low-intensity helium plasmas [158]–[160], [162, 163]. The cells, detached due to the plasma

treatment, remain viable, reattach to the plate surface and proliferate after a short incubation time. Besides the alteration of cell adhesion, a short-term exposure to the helium plasma causes a temporary cell membrane permeabilization [163] and inhibition of cell migration [160]. Longer exposure times or treatment with higher intensity plasmas induced either cell apoptosis (programmed cell death) or necrosis (accidental cell death) [158]–[160], [162]. The mode of the helium plasma-induced cell death depends on the plasma dose and irradiation conditions. Kalghatgi *et al* [156] observed similar cellular responses to plasmas generated using a DBD plasma device. Depending on its intensity and exposure time, the DBD-plasma treatment induced either apoptosis or caused necrosis in endothelial cells. In addition, authors reported an induction of cell proliferation five days after treatment with low-intensity plasmas. The observed stimulation of cell proliferation presumably results from the release of growth factors (e.g. fibroblast growth factor-2 (FGF2)) by the plasma-damaged cells.

Identification of plasma agents that induce the cellular responses described above is crucial for understanding the mechanisms of plasma–cell interactions. This information would provide a basis for designing task-specific plasmas for therapeutic applications. Experiments performed by Stoffels' group show that different plasma agents are involved in such processes as cell apoptosis induction and alteration of cell adhesion. The fact that the addition of antioxidants to the culture media did not prevent the detachment of plasma-treated cells provides evidence against the role of plasma-generated reactive oxygen species in this process [159]. Recent results presented in Stoffels *et al* [162] give additional support to this conclusion. In this study, plasma was applied to endothelial and smooth muscle cells through a porous cell culture membrane. The membrane allowed for the penetration of reactive oxygen and nitrogen species and their direct contact with cells grown on the opposite side of this membrane, while blocking charged particles and UV photons. No cell detachment was observed under these experimental conditions. However, the membrane did not prevent the plasma-induced cell apoptosis. Based on these results, the authors hypothesized that the cell detachment observed in their previous experiments [158] should be ascribed to electrostatic plasma–cell interactions. Plasma-generated reactive oxygen and nitrogen species are most probably the cause of plasma-induced cell apoptosis. Kalghatgi *et al* [156] also consider RS as the key players of plasma-induced proliferation and apoptosis in endothelial cells. The observed cell membrane permeabilization by low-intensity plasmas can be assigned to the action of the electric field (see section 6).

Summarizing the results on plasma treatment of mammalian cells published so far, different doses of low-temperature plasma can cause necrosis, apoptosis or cell detachment. Since the plasma-induced detachment of cells is a reversible process, the plasma-treated cells can be removed, transferred and reattached. This opens new possibilities in fine surgery, where a part of the tissue must be removed with high precision without damaging surrounding tissues or causing inflammation. A non-inflammatory treatment of arterial walls in patients suffering with atherosclerosis is one possible area of application of low-temperature plasmas [162]. The ability of plasmas stimulating or inhibiting cell proliferation suggests that low-temperature plasmas provide a promising tool for a localized regulation of angiogenesis [156].

5.3. Effects of non-thermal plasma treatment on malignant cells

The discovery of the pro-apoptotic capacity of low-temperature atmospheric-pressure plasmas calls attention to plasmas as a potential method of non-inflammatory anti-cancer therapy. The

removal of cancer cells by inducing apoptosis has several advantages compared with necrosis. Necrosis is generally associated with a rapid release of intracellular enzymes and cell breakdown products, which cause inflammation and damage to neighbouring tissues. During apoptosis, cells maintain their membrane integrity thus preventing the leakage of the pro-inflammatory intracellular contents. The protease activation leads to the digestion of macromolecules and fragmentation of cellular structures preparing cells for their ingestion by macrophages and adjacent cells [166].

The results of the pilot studies performed by several research groups confirmed that treatment with low-temperature plasmas is able to induce several modes of cell death including early or late apoptosis and necrosis in cultured human melanoma and hepatocellular carcinoma cells [157], [167]–[169]. Fridman *et al* [157] and Kim *et al* [168] observed signatures of apoptosis such as DNA fragmentation and release of the mitochondrial protein cytochrome *c* in malignant cells irradiated with low-intensity plasmas. The authors consider plasma-generated RS as the main cause of the apoptosis induction in these cells. Zhang *et al* [169] showed that the effect of argon–oxygen plasma on cell viability depends on the oxygen concentration. They indicated oxygen and hydroxyl radicals ($O^{\cdot}$ and $OH^{\cdot}$, respectively) as critical agents of the plasma-induced apoptosis in malignant liver cells.

An effective anti-cancer therapy should selectively trigger apoptosis in malignant cells without damaging the adjacent normal cells. The result of the comparative study of plasma effects on normal and malignant human liver cells presented in Zhang *et al* [169] showed higher susceptibility of the cancer cells to the plasma treatment. Further research is required to understand the mechanisms of the selectivity of plasma effects on cells.

6. Electric fields and living cells and tissues

6.1. History

The influence of electric fields on living cells and tissues is a direction of research which is already quite old. However, only in the eighties the number of publications in this area started to explode. This is probably triggered by public unrest regarding the dangers of mobile phone technology and high voltage lines. The scientific understanding has matured gradually as more and more detailed information became available. In this section, we do not have the intention to be complete. We will refer to a number of key publications and describe the growing scientific insight to be obtained by those.

McCaig *et al* [170] and Mamontov *et al* [171] have written comprehensive reviews on various phenomena that can occur when cells are subjected to electric fields, and they also discuss the electric fields that can be present in the human body. One of the claims is that wounded epithelia create an electric field that controls wound healing. Nerve growth is enhanced and directed by electric fields. Cells may be stimulated to move in the direction of the electric field (see the section devoted to that later). As a conclusion, the perspectives of electric fields for wound healing are discussed briefly, as well as the potential of electric fields for healing cancer. For a recent publication see [172].

6.2. The role of calcium

Calcium ions play a central role in the analysis of the interaction of electric fields and living cells. Cho *et al* [174] have performed direct measurements (epifluorescence microscopy) of the

Ca^{2+} concentration, showing a redistribution of integral membrane proteins, reorganization of microfilament structures, and a four-fold increase of the intracellular calcium ion concentration for low frequency (1–10 Hz) electric fields applied to human hepatoma cells. They attribute all this to an increased influx of Ca^{2+} through the cell membrane from the extracellular medium. Different authors [173]–[175] describe how cells can respond to voltage differences of around 0.1 mV across themselves. They attribute this to differences in the cross-membrane potential, with different behaviour of the cathode facing and the anode facing side of the cells, caused by differences in passive Ca^{2+} transport (electrophoresis or electro-osmosis). In these papers, the Ca^{2+} ions are considered to be the most important reagents in the interaction mechanisms; however, the role of the cell membrane is also already mentioned.

6.3. Cell membrane permeabilization

An overwhelming majority of the papers in the literature points to the role of the cell membrane, to be more precise, its permeabilization due to electroporation. If an electric field is present across a living cell, the potential across the cell membrane and across the intracellular membranes is modified. At the anode side of the cell, the membrane potential difference is increased. At the cathode side it is reduced. This has consequences for the transport phenomena, more precisely the calcium transport.

6.4. Measurements

Bourgignon *et al* [175] have done experiments on human fibroblast cells with high voltage pulse galvanic stimulation. The rates of protein and DNA synthesis increased by 40–60%. There is an 'optimum' voltage around 50–75 V (7.5 cm^{-1} electrode separation), which corresponds to an electric field strength of 6–10 V cm^{-1}. Cells located near the cathode of their setup grew faster. Exposure to larger fields (>30 V cm^{-1}) inhibits the protein and DNA production. As a mechanism they point at depolarization and hyperpolarization of the cell membrane. The ion channels are gated and membrane bound enzymes are activated. Forrester *et al* [176] as well as other authors [177, 178] point to modifications of the cell membrane potential, resulting in modified ion transport. They also mention the disruption of the organization of the 'bound water' at the cell membrane in connection to trans-membrane proteins such as receptors. Chang *et al* [178] find that dc fields induce reversible permeabilization of cell membrane, whereas ac fields also can induce sonic motion and structural fatigue of cell membrane. Teissié *et al* [179] have studied cell membrane permeabilization (electropermeabilization) and cell fusion. They report a critical potential difference across the cell membrane for inducing permeabilization of 200 mV.

Beebe *et al* [180] have demonstrated that short pulses in the electric field induce calcium release from intracellular stores and subsequent calcium influx through store-operated channels in the plasma membrane. Susil *et al* [181] have determined the permeabilization of the cell membrane, and the suppression or increase of the trans-membrane potential caused by cell density changes. The cell density and cell organization are very important in determining the induced transmembrane potential resulting from an electric field.

The paper by Frey *et al* [182] deserves special attention. The authors present a measurement of the membrane potential by staining Jurkat cells with Anninie-6, a voltage sensitive dye with 5 ns response time. The voltage across the anodic pole of the cell membrane rises to 1.6 V after

15 ns. This is twice the value needed for electroporation. At the cathodic side, the voltage is 0.6 V. The imposed field was 10 V μm^{-1}, so the field penetrates the cells and its organelles undisturbed. These measurements finally have shown that the effects that are assumed by other authors actually occur and can be detected.

6.5. Simulations and modelling

Binhi *et al* [183] have developed a model that predicts the also experimentally observed 'window effect': small electric fields do have an influence, larger fields do not. They suggest an ion-interference mechanism: the quantum interference of ions bound within proteins. Their model is based on solving the Schrödinger equation.

Several authors, e.g. [184]–[186], have modelled the cell membrane and the electroporation processes associated with electric fields. Separate models have been developed for the electrolytic behaviour with conductive and dielectric interactions, and a membrane–electrolyte interaction model, a multicellular model with active and passive interactions, a membrane channel population model, a membrane electroporation model and Kirchoff's laws are all coupled. All this is used to predict the behaviour of biological systems upon exposure to electric fields.

Joshi *et al* [187] have performed a model study of electroporation, based on the Smoluchowski equation. They demonstrate that a minimum pulse duration is required for irreversible breakdown of the cell membrane. These models have been refined and extended by the same authors [188], now encompassing a self-consistent model of the electroporation dynamics. A coupled scheme of the Laplace, Nernst-Planck and Smoluchowski equations is used. They find that there is a finite time delay for pore formation, with a voltage overshoot. Pore closing is generally slower (10^{-1} s). Hu *et al* [189] have performed molecular dynamics simulations of cell membranes exposed to nanosecond pulsed electric field (nsPEF). They predict pore formation within 5–6 ns, tending to start from anodic side of the electrically stressed membrane.

6.6. DNA

A few authors discuss the connection between electric fields and DNA. Mamontov *et al* [171] discuss the stimulation of a three-fold increase of cell mitosis in a 50 Hz electric field. As a first-order explanation, they suggest that the cells pass quicker through the G_2 period in the mitosis process. Bourgignon *et al* find an increase of the DNA synthesis rate by 40–60% for a narrow process window (about 10 V cm^{-1}). Larger fields have less effect. Stickley *et al* [177] have observed DNA damage in non-adherent cells caused by nanosecond pulses of very high voltages. Adherent cells do not demonstrate this behaviour, except for mouse 3T3 cells.

Wang *et al* [190] have studied a sort of reverse of wound healing: the inhibition of the proliferation of lens epithelial cells. They claim that this is a cause for secondary cataract. They also indicate that this phenomenon may be related to modifications in the transition between the G_1 to the S phase in mitosis.

6.7. Cell movements

In some cases, electric fields can stimulate the cells to move and align themselves either parallel or perpendicular to the direction of the electric field. Zimmerman *et al* [191, 192] point to

polarization of the cell membrane as primary cause for enhanced cell fusion and orientation. They have observed non-spherical cells orienting themselves in the presence of an electric field. Depending on the frequency, transport and orientation of cells occur either parallel or perpendicular to the electric field. Partly, this is an electrostatic effect (induced polarization of the cell, and then electrostatic force).

Brown *et al* [193], just as Zimmerman *et al* [191, 192] have detected electric field induced locomotion of 3T3 fibroblast cells. They claim that this process is calcium independent. Galvanotaxis involves the lateral redistribution of plasma membrane glycoproteins, which are involved in cell-substrate adhesion.

Onuma *et al* [194] have observed cell elongation and alignment in the direction of the field ($10\,\mathrm{V\,cm^{-1}}$). A preferential position shift towards the cathode occurs, which could be inhibited by a calcium channel blocker (D-600). An increase in the intracellular calcium concentration was measured. As an explanation they suggest a depolarization of the cell membrane on the cathode side, which causes a larger calcium influx, which in turn causes depolymerization of the actin cortical meshwork, which then contracts and 'pulls' the cell forward. This is assisted by activation of the actomyosin system. Combined, these phenomena cause the cell to retract from the anode directed region and move towards the cathode.

6.8. Ultra-short pulses

Karl Schoenbach of Norfolk, Virginia, is the 'founding father' of the application of nsPEFs. He and his co-authors have published a large number of papers, in which they shed light on many of the underlying mechanisms in the interaction between the electric field and living cells ([194]–[206] are good examples, but many more can be found in the literature).

The consequences of changing the pulse duration were investigated in [195]. As the pulse duration decreases, the electroporation of the cell membrane also decreases and the poration of intracellular membranes increases. If the electric fields are sufficiently high, nsPEF can induce apoptosis in human and mouse cells. Using flow cytometry and immunofluorescence microscopy it is possible to show damaged DNA after nsPEF exposure [196], but also a reduced tumour growth in a mouse model was observed. If pulses are short enough, cell membrane electroporation does not occur (the membrane potential adapts slower than the pulse duration), but the inner cell structure is modified.

Very high fields ($>50\,\mathrm{kV\,cm^{-1}}$) have the potential to affect transport processes across intracellular membranes and may be used for gene transfer into cell nuclei [197]. They can also trigger apoptosis for cancer treatment. Even more intense pulses of $300\,\mathrm{kV\,cm^{-1}}$ [198] and 10 ns induce modifications of the intracellular structures: breaching of intracellular granule membranes without inflicting damage to the cell membrane, abrupt rises in intracellular calcium concentrations and enhanced gene expression. Submicrosecond pulses induce apoptosis.

Escherichia coli can be killed if the pulses are short enough [199] and other types of bacteria also die due to nsPEF. Furthermore, aquatic nuisance species (Zebra mussels, Daphnia and Brine shrimps) can be stunned. This indicates a potential for biofouling control.

6.9. Cancer treatment and wound healing

Kirson *et al* [185] have applied ac fields to inhibit the proliferation of various cancer cells (malignant melanoma, glioma, breast carnicoma and lung carnicoma). Clinical tests indicate a

halving of the rate of progression of recurrent glioblastoma. Hardly any side effects have been detected >70 months after treatment. Nuccitelli *et al* [200] have applied nanosecond pulsed electric fields (nsPEF) of $>20\,\mathrm{kV\,cm^{-1}}$ and 30 ns rise time. They find that tumour cell nuclei rapidly shrink, and tumour blood flow stops. Melanomas are observed to shrink by 90% within two weeks following treatment. A second treatment then results in complete remission. These pioneering papers clearly indicate that electric fields have good potential in treating cancer.

Goldman *et al* [207] have studied the influence of electric fields on wound healing. They find a narrow window ($37–50\,\mathrm{V\,m^{-1}}$, 10 Hz), within which the cell proliferation rate of human dermal fibroblasts is enhanced. Dubé *et al* [208] have studied wound healing on *in-vitro* cultured skin samples. An enhanced proliferation of epithelial cells is observed, an increased cell migration and collagen synthesis.

7. Summary and conclusions

Plasma Health Care has developed in the last few years into an innovative and growing field of research and development. It combines a number of fields—physics, chemistry, engineering, biology, microbiology and medicine—into an extensive multidisciplinary research effort.

Current activities relate to the design of specialized plasma sources and delivery techniques, to understanding the processes that lead to fungicidal and bactericidal effects without harming mammalian cells, to maintain prescribed safety standards and to investigate the processes that are of relevance in medicine and hygiene. At the same time, more 'adventurous' paths are pursued—the search for plasma designs that produce apoptosis (programmed cell death) in cancer cells, whilst leaving normal cells unaffected (cancer cells have different mechanical properties with respect to normal cells—there is a change in their cytoskeleton, which makes them more compliant—a fact that plasma treatment may exploit), the role of the cell cycle on plasma treatment efficacy, the possibility of molecular drug delivery (including short-lived locally produced species in the non-equilibrium plasma-chemical processes), investigations of resistance development of bacteria to plasma treatment and the optimization of antibacterial plasma designs, etc.

The principally new elements in plasma health care are the molecular delivery of RS (and the corresponding ability to access even micron-sized regions), the possible use of ions as active agents (drugs), the combination of charging by electrons and the induced electric fields, leading to an increased permeability of cell walls for drug delivery, the specific production of RS inside cells leading to a targeted chemistry (e.g. Fenton's reaction) and, of course, the possibility to 'design' the plasma with respect to various abundances and combinations of RS.

These new elements alone will allow for a broad scope of future research—both fundamental and applied. The NJP Focus Issue highlights recent studies in what promises to develop into an exciting and important multidisciplinary research field with a huge application potential for the benefit of mankind.

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