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Plasma medicine—current state of research and medical application

K-D Weltmann and Th von Woedtke

Leibniz Institute for Plasma Science and Technology (INP), Greifswald, Germany

E-mail: woedtke@inp-greifswald.de

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Abstract

Plasma medicine means the direct application of cold atmospheric plasma (CAP) on or in the human body for therapeutic purposes. Further, the field interacts strongly with results gained for biological decontamination. Experimental research as well as first practical application is realized using two basic principles of CAP sources: dielectric barrier discharges (DBD) and atmospheric pressure plasma jets (APPJ). Originating from the fundamental insights that the biological effects of CAP are most probably caused by changes of the liquid environment of cells, and are dominated by reactive oxygen and nitrogen species (ROS, RNS), basic mechanisms of biological plasma activity are identified. It was demonstrated that there is no increased risk of cold plasma application and, above all, there are no indications for genotoxic effects. The most important biological effects of cold atmospheric pressure plasma were identified: (1) inactivation of a broad spectrum of microorganisms including multidrug resistant ones; (2) stimulation of cell proliferation and tissue regeneration with lower plasma treatment intensity (treatment time); (3) inactivation of cells by initialization of programmed cell death (apoptosis) with higher plasma treatment intensity (treatment time). In recent years, the main focus of clinical applications was in the field of wound healing and treatment of infective skin diseases. First CAP sources are CE-certified as medical devices now which is the main precondition to start the introduction of plasma medicine into clinical reality. Plasma application in dentistry and, above all, CAP use for cancer treatment are becoming more and more important research fields in plasma medicine. A further in-depth knowledge of control and adaptation of plasma parameters and plasma geometries is needed to obtain suitable and reliable plasma sources for the different therapeutic indications and to open up new fields of medical application.

Keywords: cold atmospheric plasma, plasma medicine, plasma jet, dielectric barrier discharge

(Some figures may appear in colour only in the online journal)

1. Introduction

For more than ten years, plasma medicine is emerging worldwide as a new field of medical research at the interface between plasma physics and life sciences. In general, plasma medicine means the application of physical plasma for medical purposes. Whereas established electro-surgical techniques such as argon plasma coagulation (APC) or coblation are mainly based on bio-destructible plasma effects, actual research and application of plasma medicine is focused on selective, at least partially non-lethal, possibly stimulating plasma effects on living cells and tissue (von Woedtke *et al* 2013a).

The development of suitable and reliable plasma sources for different medical applications requires an in-depth knowledge of plasma physics and chemistry on the one hand and its impact on biological systems like microorganisms, cells and tissue on the other. The ultimate physical and technical objective is to design easily-controllable plasma devices specifically adapted to different demands (indications) in medical therapy.

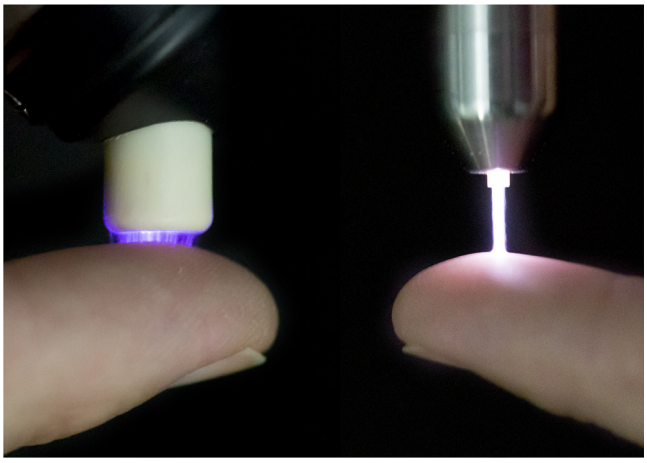

Figure 1. Volume DBD (left) and plasma jet (right) in contact with finger tips.

2. Basics of plasma medicine

2.1. Cold atmospheric plasma for medical applications

For the application directly on or in the human (or animal) body for therapeutic purposes plasmas are needed that

- operate stable and reproducible under open atmospheric conditions, and
- are cold (<40 °C) at the tissue contact zone to avoid thermal destruction.

Most cold atmospheric pressure plasmas (CAP) for biomedical applications are generated by applying electrical energy to a not directly biologically effective gas (argon, helium, oxygen, nitrogen, air, or mixtures thereof). The main part of the applied energy goes into the production of energetic ('hot') electrons whereas the majority of gas atoms, ions and molecules remain in a low-energetic state resulting in a low plasma temperature. Excited and ionized atoms or molecules interact with other atoms or molecules of the working gas and—because of the open atmospheric conditions—with neighbouring media (above all atmospheric air, but also liquids and surfaces) resulting in the generation of reactive species with biological potential. Additionally, excitation processes result in emission of electromagnetic radiation (UV/VUV, visible light, IR/heat, electric fields).

During the last years, two basic plasma device principles were established in research and are also used in first practical applications (Weltmann *et al* 2010, Isbary *et al* 2013a, von Woedtke *et al* 2014).

With the volume dielectric barrier discharge (DBD), plasma is ignited in the gap between an isolated (dielectric) high-voltage electrode and the tissue to be treated, i.e. human body is serving as counter electrode (figure 1, left). Most of these devices are using atmospheric air as working gas.

Atmospheric pressure plasma jets (APPJ) consist of a gas nozzle equipped with one or two electrodes. The plasma is ignited inside the nozzle and transported to the outside as well as to the object to be treated by a flow of a pre-assembled working gas (figure 1, right). Plasma jets can differ in electrode configuration, type of gas, and applied electrical parameters (Winter *et al* 2015). In remote plasmas, the plasma is potential free (which is for some medical indications advantageous and necessary) and consists of relaxing and recombining active species from inside the nozzle. In some active plasma jets, the targeted substrate forms a second or third electrode and the expanding plasma contains free and high energetic electrons, i.e. the plasma is not totally potential free.

From a practical point of view, both concepts have advantages and disadvantages. With a DBD, the covering of large areas is possible more easily than with the plasma jet which is more useful for well-directed spot-like treatments. DBDs mostly use atmospheric air as working gas. This is an advantage at first glance because no additional gas supply equipment is needed. However, with plasma jets that use pre-assembled working gases, the working gas composition is much better defined and can kept stable. Moreover, controlled working gas variations are possible if needed to trigger specific biological effects. With DBD, any variations of environmental air conditions, e.g. humidity, might be more significant compared to the plasma jet (see below). The distance between plasma-generating device and target (e.g. skin or wound surface) is much more critical for the volume DBD than for the plasma jet. Last but not least, because of the human tissue functioning as counter electrode (e.g. skin or wound surface), any variation of its conductibility might influence the plasma ignition and consequently has to be taken into consideration. A robust process window has to be guaranteed for reproducible applications in medicine. Nevertheless, all these advantages and disadvantages have to be investigated more in detail to estimate its impact for practical applications. From the present point of view, it is expected that both DBDs and plasma jets will find their specific fields of application in medicine.

2.2. Biological effects of cold atmospheric plasma

In the initial period of plasma medicine, a huge number of studies were done to characterize several effects of CAP on microorganisms and mammalian cells *in vitro*. Despite the use of different plasma sources, working gases, experimental setups, cell types, microorganism strains, etc. some general biological plasma effects have been described repeatedly (Fridman *et al* 2008, Stoffels *et al* 2008, Dobrynin *et al* 2009, Morfill and Zimmermann 2012, von Woedtke *et al* 2013a, 2014). Among them, especially its potential to inactivate a broad spectrum of microorganisms including multidrug resistant ones (Daeschlein *et al* 2014, Napp *et al* 2016) and its potential to stimulate cell proliferation and angiogenesis and consequently to promote tissue regeneration and wound healing (Barton *et al* 2013, Hasse *et al* 2014, 2015, Haertel *et al* 2014) was identified to be very useful for medical purposes. Moreover, it was demonstrated that in mammalian cells and especially in cancer cells the programmed cell death (apoptosis) can be initialized by higher plasma treatment intensities (treatment times) which opens up further application fields in oncology (Partecke *et al* 2012, Schlegel *et al* 2013).

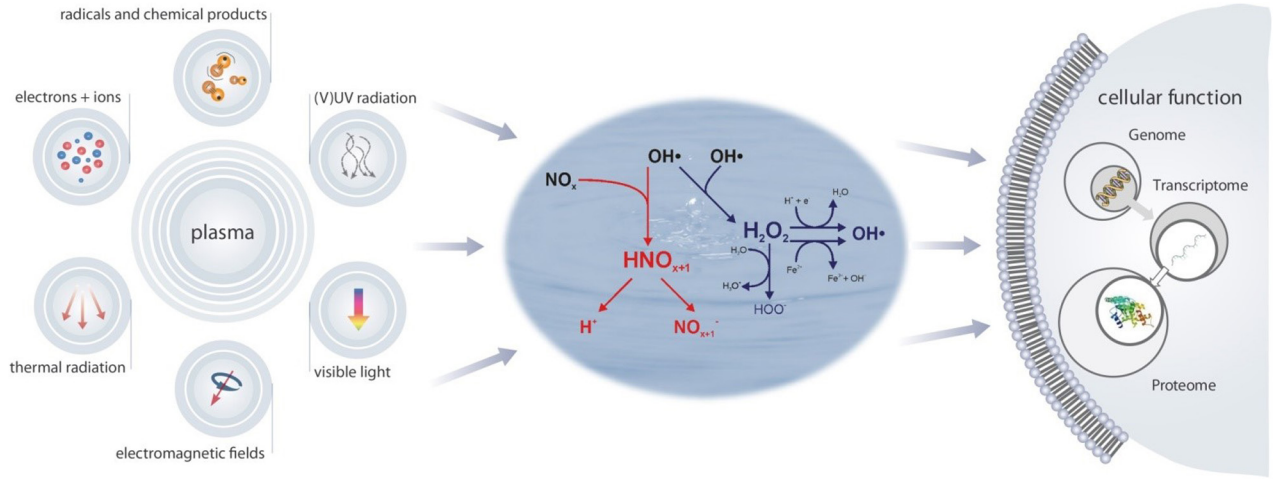

Figure 2. Schematic of transmission of biological plasma effects via liquid phases.

Based on this basic knowledge on general reactions of living cells and tissue to CAP treatment, more and more basic research has been done to understand and explain mechanisms of these biological effects using highly sophisticated cell biological and molecular biological techniques.

According to the actual state of knowledge, biological effects of CAP are based on two major fundamental principles (figure 2):

1. Biological plasma effects are significantly caused by plasma induced changes of the liquid environment of cells.
2. Reactive oxygen and nitrogen species (ROS, RNS) that are generated in or transferred into liquid phases play a dominating role in plasma-induced biological responses.

According to the actual knowledge other plasma components like UV radiation or electrical fields/electrical current are considered to play additional roles in the active plasma 'cocktail'. However, UV part of CAP is estimated to have low or no direct biological effects because typically low doses are emitted by plasma devices designated for medical use. But its supporting role in reactive species generation by photochemical activity has to be taken into consideration. Electrical fields or current, respectively, reaching living tissue depends strongly on type of discharge and therefore will cause varying direct biological effects. Much more research is needed in this field to finally enlighten the role of this plasma compound for its possible direct part in biological and medically relevant plasma action but also for its role in the generation or support of action of other plasma compounds, above all of reactive species (Darny *et al* 2015).

In other physical technologies for medical therapies like phototherapy, radiotherapy, laser therapy, etc, the effects are mainly based on direct irradiation activity supplemented by more or less important secondary effects of generation of reactive species via irradiation energy absorption of the liquid environment. In contrast to that, in plasma therapy secondary generated reactive species in the liquid phase are the predominant players which will enable more 'soft' and controllable therapeutic applications.

Table 1. Biologically important reactive oxygen and nitrogen species.

Reactive oxygen species (ROS)	Reactive nitrogen species (RNS/RONS)
Superoxide: O_2^- •	Nitric oxide: $^\bullet$NO
Hydrogen peroxide: H_2O_2	Nitrogen dioxide: $^\bullet NO_2$
Hydroxyl radical: $^\bullet$OH	Peroxynitrite: $ONOO^-$
Singlet oxygen: $^1O_2$	
Ozone: O_3	
Organic radicals: $RO^\bullet$, $RO_2^\bullet$	

The ROS and RNS produced as a result of plasma-liquid interactions to act on cells and tissues (table 1) are the same as occur in regular physiological and biochemical processes in the body (Graves 2012, 2014).

Based on this fundamental insight, the large field of redox biology can be used now as scientific basis to explain biological effects of CAP. Moreover, using insights from redox biology a precise risk estimation was possible. Because of the regular occurrence of ROS and RNS in cell biological processes mammalian cells have protective mechanisms to save from reactive species concentrations going beyond physiological levels leading to oxidative stress with severe consequences, e.g. genotoxic DNA changes. Detailed investigation using well-established experimental procedures could demonstrate that application of cold atmospheric plasma does not cause increased risk for genotoxicity (Boxhammer *et al* 2013, Kluge *et al* 2016, Wende *et al* 2016).

On the other hand, by control of reactive species generation by plasma and investigation of its biological impacts more detailed insight will be gained about detailed mechanisms of biological plasma effects.

3. Diagnosis and variation of reactive species production by plasma

Besides technical parameters like electrode arrangement, geometry and input power, generation of reactive species in cold atmospheric pressure plasma devices is mainly

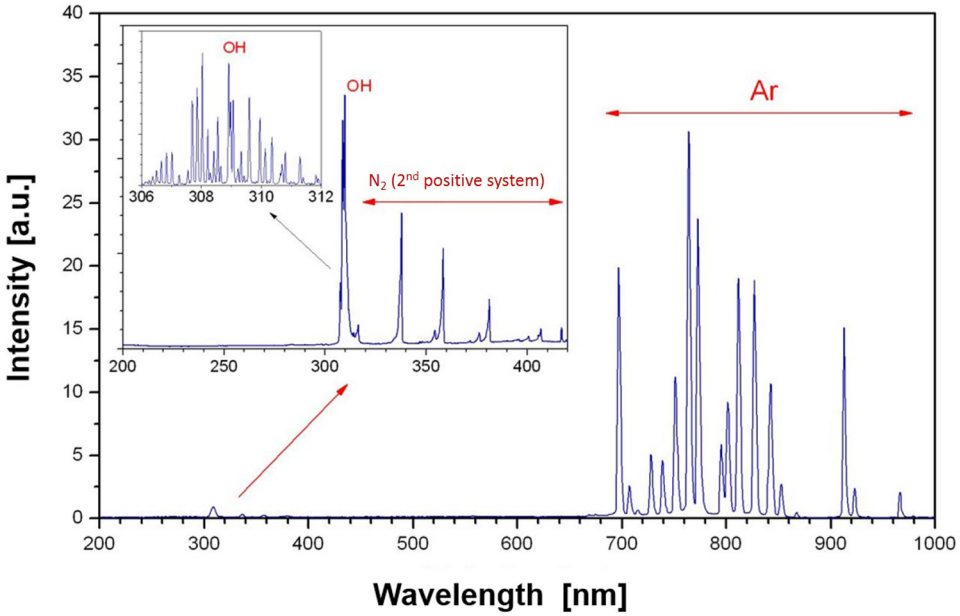

Figure 3. Optical emission spectrum of the Ar-driven cold atmospheric pressure plasma jet kINPen.

determined by two components: the working gas used for plasma ignition and the atmospheric air working environment of the plasma.

The argon-driven cold atmospheric pressure plasma jet kINPen developed by INP Greifswald, Germany (Weltmann *et al* 2009), is one of the most investigated cold atmospheric pressure plasma sources. Therefore, the data and comments of this paper are mainly based on selected experimental studies using this device.

Because of the use of argon (Ar) as pre-assembled working gas with this kind of plasma jet the influence of working gas on plasma species generation is easily controllable.

For comprehensive plasma characterization, different plasma diagnostic techniques including electrical measurements, optical and spectroscopic techniques and flux analysis techniques are established (Bruggeman and Brandenburg 2013). Techniques like UV and FTIR absorption spectroscopy, TALIF spectroscopy and mass spectrometry are used to identify and quantify ions and reactive species in the plasma and to track its way from gas to liquid phase (Dünnbier *et al* 2013, Pipa *et al* 2012, Reuter *et al* 2012a, Winter *et al* 2012, 2014, Schmidt-Bleker *et al* 2014). Because of its comparatively easy manageability, optical emission spectroscopy (OES) is primarily used for plasma diagnostics to get an overview of plasma characteristics even if its information value is restricted to excited and radiant species.

Assessing the optical emission spectrum of the Ar-driven cold atmospheric plasma jet kINPen (figure 3), besides the dominating lines of excited argon atoms between 700 and 900 nm the nitrogen emission lines between 330 and 420 nm as well as the OH emission at 309 nm are of particular interest. Using pure Ar as working gas, nitrogen (N_2) comes from the atmospheric air environment and is, together with atmospheric oxygen (O_2) a precursor of production of NO which plays a variety of important biological roles, among others in wound healing. The generation of NO production by plasma can be controlled by air admixture to the working gas.

As it is demonstrated in figure 4, left, increasing parts of air admixture to the working gas Ar results in both increasing intensities of nitrogen emission (330 and 420 nm) and increasing emission of the γ-bands of NO between 200 and 300 nm. Consequently, production of NO radicals can be controlled by variation of air admixture to the working gas Ar (Pipa *et al* 2012).

At the same time, emission of OH at 309 nm is reduced with increasing air admixture (figure 4, left). However, as pictured in figure 4, right, OH emission at 309 nm can be intensified by systematic humidification of the working gas argon. Because the highly reactive OH radical and its reaction partner hydrogen peroxide (H_2O_2) are playing important roles in several biological processes, it is very useful to control its generation by CAP. With further experiments it could be demonstrated that the feed gas humidity has a much larger effect on OH emission than the humidity of the ambient air (Winter *et al* 2013). This insight is highly important for two reasons. First, with a plasma jet using a pre-assembled working gas like argon, generation of OH and supposable that of other reactive species can be kept constant by defined working gas characteristics. Secondly and possibly much more important, the influence of surrounding air humidity as one of the most changing environmental condition is of less importance if a plasma device with a pre-assembled working gas is used. Presumably it is much more important if the plasma is generated in environmental air, e.g. with DBD based devices.

Based on such insights about possibilities of control and manipulation of reactive species generation by CAP, as a next step this can be specifically used to identify specific biological effects of single reactive species. It was demonstrated that increasing humidity and subsequently increasing OH concentration results in lower viability of skin cells *in vitro* (Winter *et al* 2014).

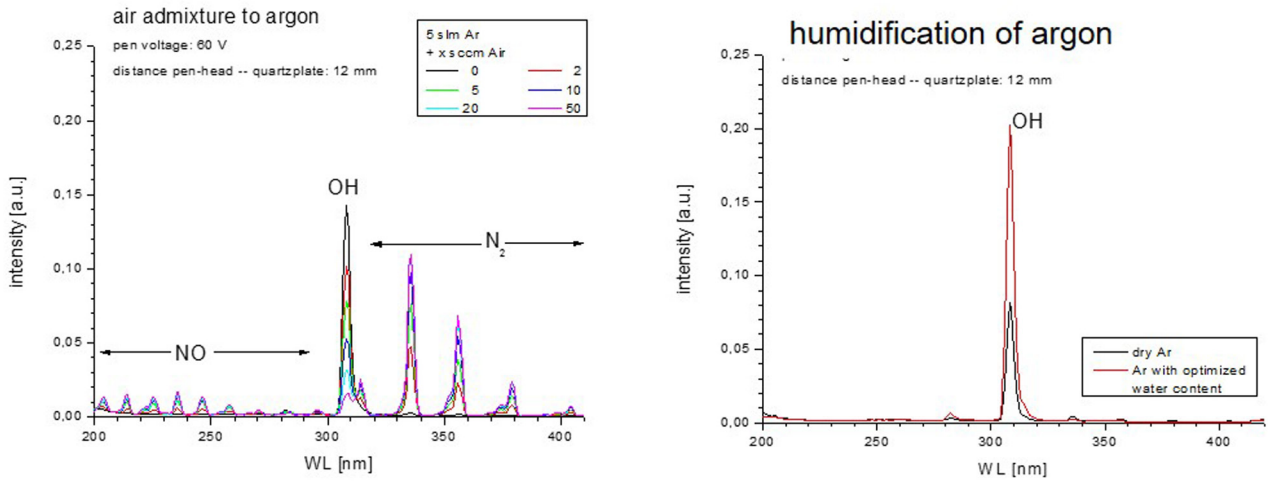

Figure 4. Optical emission spectra of the Ar-driven cold atmospheric pressure plasma jet kINPen: admixture of 5–50 sccm air to the 5 slm Ar working gas flow (left) and difference of dry and humidified Ar (right).

In another experimental series using a newly designed technique to manipulate the immediate environment of the plasma jet by using a shielding gas curtain (Reuter *et al* 2012b), it was found that both ROS and RNS are necessary for effective bacteria inactivation in liquid environment whereas the cytotoxic effect on skin cells is dominated by the ROS generated by the plasma jet (Reuter *et al* 2012c, Jablonowski *et al* 2015). This can be taken as a clear indication that any variation of the plasma composition may result in different biological responses. The aim of such variation of plasma's reactive species composition is not only to understand the specific role of different reactive species for different biological effects but from a practical point of view to 'tune' the plasma to realize specific and selective therapeutic effects, e.g. selective inactivation of bacteria while maximally sparing the surrounding healthy tissue. However, such investigations of variable plasma composition and its biological as well as medical impact are on its beginning and need much more research before a practical use will be possible.

In all these research activities, plasma-liquid interactions have been becoming more and more important in recent years (Jablonowski and von Woedtke 2015). As already stated, biological plasma effects are primarily transmitted via liquid phases. It was demonstrated repeatedly that liquid chemistry is influenced by plasma treatment (Oehmigen *et al* 2011, van Gils *et al* 2013, Wende *et al* 2015). Starting with very complex chemical interactions in the plasma phase, reactive species are introduced into the liquid or are generated there by secondary reactions. All these chemical interactions are supported more or less by other plasma components like irradiation and electric fields. Identification of reactive species in liquids is considered to be one of the keys for detailed understanding of biological plasma effects on a molecular level. However, these highly reactive low molecular reactive species are very hard to analyse by classical wet-chemical methods. This is caused by its short life time on the on side, but also by the fact that all these species are interacting with each other. Conventional chemical analytics are mostly based on quantitative consumption of the molecule to be diagnosed, e.g. by a dye. Consequently, the interaction between the reactive species might be disturbed by the analytical process itself. Highly sophisticated analytical methods like electron paramagnetic resonance (EPR) spectroscopy will help to further investigate liquid chemistry in response to CAP treatment (Tresp *et al* 2013). However, most of the liquid analytics up to now are realized in 'simple' liquids like water or physiological saline only. To come closer to the *in vivo* situation and to include also the influence of components of vital environment in living tissue, e.g. proteins, lipids, salts, etc. much more complex model liquids have to be investigated which will make also the analytical challenges much more complex. Eventually, for investigation of the complex reaction chains from plasma / gas phase via liquid environment of cells to different and specific cellular and tissue responses the use of mathematical models will become more essential (Orazov *et al* 2012, Babaeva *et al* 2014).

In general, it was found with several different CAP devices that all these very complex plasma-liquid interactions finally result in a decrease of pH value and in production of nitrite (NO_2^-), nitrate (NO_3^-), and hydrogen peroxide (H_2O_2) in plasma-treated liquids. Occurrence of these stable and easy to detect species in liquids can be generalized for different plasma sources. Therefore, as a first approach these species are considered to be representative markers for complex plasma-liquid interactions and allow conclusions on main reactive processes induced by CAP sources. pH change (acidification) is an indicator for the reactive (biological) environment, H_2O_2 represents ROS chemistry with special reference to OH and superoxide radical, and NO_2^- and NO_3^- are final products of reactions related to NO and indicate occurrence of RNS/RONS chemistry in general.

Even if it was demonstrated repeatedly that pH changes as well as generation of H_2O_2, NO_2^- and NO_3^- are not directly responsible for plasma-induced biological effects transmitted via liquid changes, its concentration or relationship to each other can be at least partially correlated with several biological effects and allows rough estimations of biological performance of plasma sources.

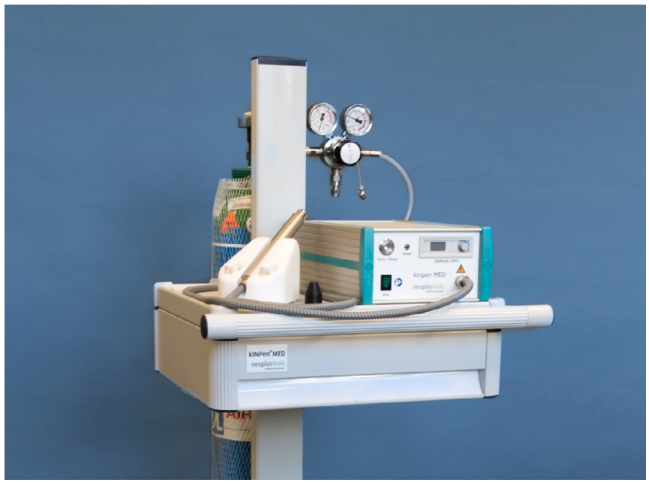

Figure 5. The cold atmospheric plasma jet device kINPen MED based on the research results from INP Greifswald (neoplas tools GmbH, Greifswald, Germany); dimensions of supply unit: 330 mm × 180 mm × 105 mm.

4. Medical application of cold atmospheric plasma

Since 2013, first CAP sources are CE certified as medical devices, mainly for the purpose of treatment of chronic wounds as well as pathogen-based skin diseases. One of it is the argon-driven cold atmospheric plasma jet kINPen MED (neoplas tools GmbH, Greifswald, Germany; figure 5), which is based on comprehensive physical, biological, pre-clinical and clinical characterization (Weltmann *et al* 2009, Bekeschus *et al* 2016).

The high potential of the Ar-plasma jet for medical applications particularly in the field of tissue regeneration, wound healing and skin decontamination and also the safety of its application has been proved by several clinical case reports, clinical trials and applications in animals (Daeschlein *et al* 2012a, 2012b, Metelmann *et al* 2012, 2013, Kramer *et al* 2013, Klebes *et al* 2015, Ulrich *et al* 2015). The routine application in medical practice in first clinics as well as doctor's offices has started. Very promising results are reported especially in the treatment of long-lasting chronic and infected wounds. Currently, it is predominantly applied as last resort in cases where conventional treatment fails. However, in these cases a re-start or acceleration of wound healing process in more than 80% is reported as a preliminary result. Especially its effectivity in eradication of antimicrobial resistant bacteria (e.g. MRSA) is emphasized by the users.

Another jet-like CAP source is the microwave-driven Ar-plasma torch MicroPlasSter, now offered as SteriPlas (ADTEC, Hunslow, UK). Long-term clinical application experience with this device was mainly focused on reduction of bacterial load of chronic wounds (Isbary *et al* 2010, 2012, 2013b, Heinlin *et al* 2013).

PlasmaDerm (CINOGY GmbH Duderstadt, Germany) is a well-investigated medical device based on a volume DBD working with atmospheric air for plasma generation (Brehmer *et al* 2015).

Based on this preclinical and clinical experience of recent years it can be stated that cold atmospheric plasma for wound healing is on its way to becoming clinical routine. Now it is mandatory to prove and consolidate plasma performance in therapeutic practice with focus on wound healing and dermatology by ongoing practical application. More systematic clinical trials are necessary to meet the demands of evidence based medicine.

Another important field of plasma application with a high practical potential is dentistry (Kim *et al* 2013, Cha and Park 2014). Besides wound healing especially disinfection of tooth root canal (Jablonowski *et al* 2013, Herbst *et al* 2015), treatment of dental implants (Teixeira *et al* 2012, Duske *et al* 2015) or therapy of intraoral infections (Preissner *et al* 2016) are promising fields of application. However, despite of a huge research effort during the last years, relevant clinical application of CAP in dentistry is rarely known until now.

Furthermore, a highly topical field of basic and preclinical research is CAP application in cancer therapy (Schlegel *et al* 2013, Ratovitski *et al* 2014, Hirst *et al* 2016). This is based above all on the fact that CAP is able to induce programmed cell death (apoptosis) in cancer cells and that these cells seem to be much more sensitive for CAP treatment compared to non-malignant cells (Partecke *et al* 2012, Weiss *et al* 2015). First animal studies could demonstrate anti-cancer effects of CAP *in vivo*, too (Keidar *et al* 2011, Vandamme *et al* 2012). This opens up new possibilities of supportive CAP application e.g. in surgical or radiative cancer eradication as well as in palliative cancer therapies (Metelmann *et al* 2015, Seebauer *et al* 2015).

Besides these large fields of basic, pre-clinical and clinical research in plasma medicine, further fields of medical plasma use like ophthalmology (Martines *et al* 2013) or plastic and aesthetic surgery are investigated.

Another new field of plasma medicine somewhat distant from direct plasma application is the use of plasma-activated liquids (e.g. PAM, plasma activated media) (von Woedtke *et al* 2013b). In connection with basic research on the role of liquid phases for the transmission of biological plasma effects it was found that by plasma treatment liquids can get biological activity. Actual research in this field is mainly focussed on the applicability of such plasma-activated liquid media to inactivate cancer cells e.g. in the case of disseminated tumours in the abdomen (Utsumi *et al* 2013, Tanaka *et al* 2015).

5. Further prospects and challenges of cold atmospheric plasma in medicine

5.1. Application adapted plasma devices

For further development of plasma medicine as a self-contained field of medical device technology like laser medicine, besides the necessity of further research in life sciences several physical and technical challenges have to be addressed.

Most of the plasma sources described and tested up to now are useful for medical applications on 'open' surfaces, e.g. on skin or wounds or during open surgery. Besides the further improvement of 'plasma tuning' for a more specific plasma activity as stated already, adapted geometries for better manageability and reachability of specific places of application like dental and visceral cavities have to be realized.

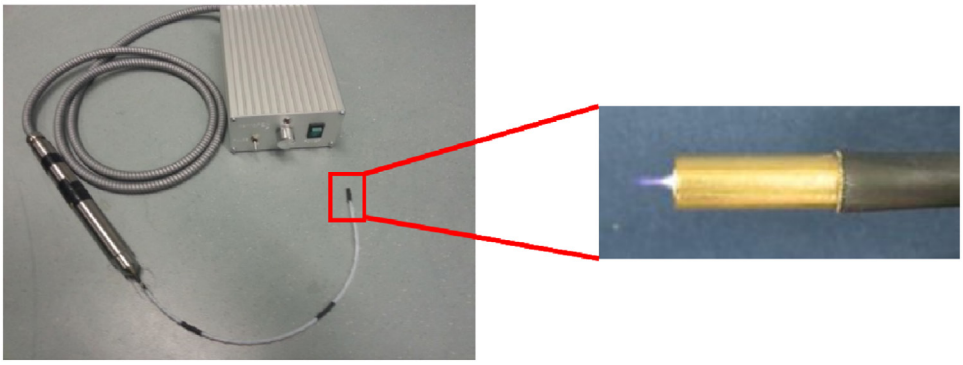

Figure 6. Prototype of a flexible catheter-like cold atmospheric Ar plasma jet device based on kINPen technology (INP Greifswald, Germany); outer diameter of the catheter 4 mm.

Figure 7. Prototype of a cold atmospheric Ar plasma jet device designated of dental applications based on kINPen technology (INP Greifswald, Germany); dimensions of supply unit (left picture): 330 mm × 180 mm × 105 mm.

Endoscopic plasma applications are realized already in electrosurgical application like argon plasma coagulation (Manner *et al* 2008). But also for cold atmospheric plasma there are promising concepts for endoscopic applicable plasma devices (Robert *et al* 2013). Besides applications in gastroenterology also the field of pneumology will be envisaged. A prototype of a flexible plasma device based on the technology of the Ar-driven cold atmospheric pressure plasma jet kINPen is available and will be tested with specific regard for antibacterial application in line with the treatment of pulmonary tuberculosis (figure 6).

A similar problem of reachability to intricate anatomic regions is also given in dentistry. Here, hand-held dental plasma devices are required that are manageable in the same manner like regular dentist's instruments. Figure 7 demonstrates a prototype of an Ar-driven cold atmospheric pressure plasma jet designated for further development as dental instrument, again based on the kINPen technology.

The simple downsizing and bending of the head of the kINPen appears easy at first glance but, because of the necessary wiring of high voltage into the tip of the device several innovative physical and technical approaches including plasma modelling had to be found to come to this technical solution and to meet successfully the electromagnetic compatibility tests (EMC).

Finally, one of the most important actual challenges in plasma application in medicine is the problem of large area treatment. Above all in wound healing and dermatological treatments, very often large areas have to be covered by cold atmospheric plasma. The area of high-voltage electrode of DBDs can be extended in that way that a covering of larger skin regions or wounds is possible. Because under practical operating conditions not always flat surfaces are to be treated, flexible or specifically shape-adapted electrode configurations have to be designed (Weltmann *et al* 2012). One approach is the use of high voltage electrode arrays on flexible carrier materials to generate a surface DBD (figure 8).

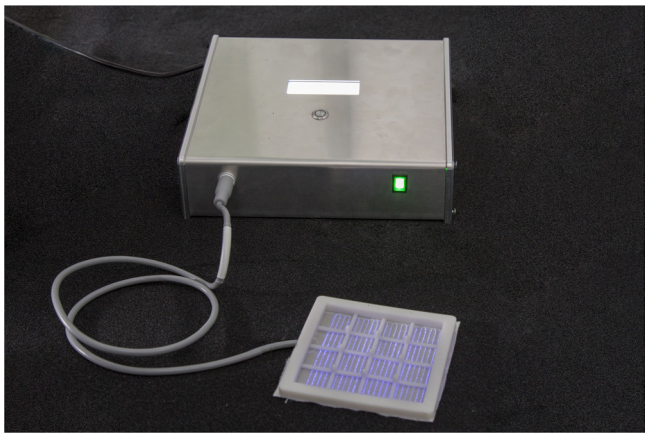

Figure 8. Prototype of a flexible flat textile surface DBD-based plasma device (INP Greifswald, Germany); dimensions of the pad: 10 × 10 cm.

In this device, similar to a wound dressing pad, the plasma is generated on the surface of the electrode configuration (based on textile or silicon based material) using environmental air

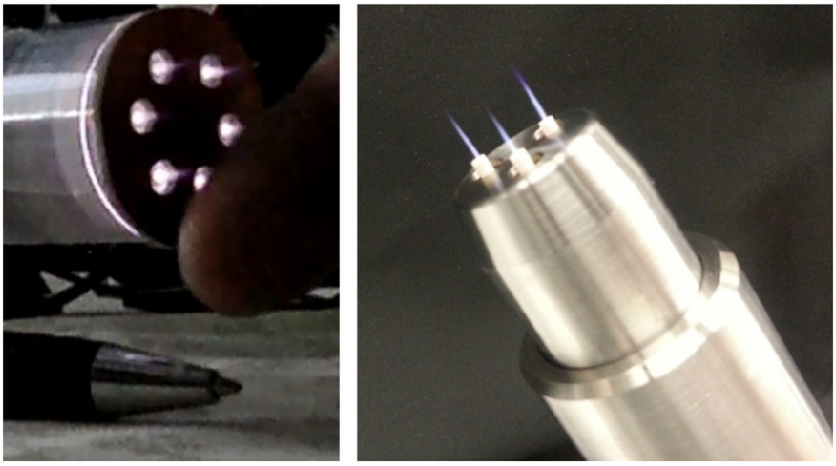

Figure 9. Prototypes of a cold atmospheric Ar plasma jet arrays based on kINPen technology (INP Greifswald, Germany).

for plasma ignition. For application, it has to be brought in close vicinity to the surface to be treated (skin, wound) but the surface does not serve as counter electrode like with the volume DBD. Therefore, any variations of the conductibility of the surface to be treated are of less importance. However, possible influences of variations in humidity of the air in the gap between plasma generating electrode structure and treated surface have to be considered as it is necessary for volume DBDs.

As it was already discussed, this last mentioned problem is not so evident in plasma jets. To increase its effective area, different setups of plasma jet arrays were designed enabling a parallel operation of multiple jets (Cao *et al* 2010, Kim *et al* 2012) (figure 9).

To put three or six or even more plasma jets into operation in close vicinity to each other with well-defined and above all stable operating parameters, innovative solutions had to be found to avoid mutual influences.

Generally, it has to be stated that reliable plasma sources are the key issue for the development of new technologies and applications also in medicine. One of the main prerequisites not only for acceptance of plasma medicine in the medical doctor's community but also to get strong criteria to distinguish scientific plasma medicine from obscure players cropping up again and again also under the level of plasma medicine is a sound and reputable scientific characterization before any medical application. This is the general basis at INP Greifswald for interdisciplinary or translational research (figure 10).

5.2. Comparability of plasma devices, monitoring and control of plasma performance

However, there is a wide variety of different plasma devices worldwide investigated which are designated for medical use. However, data about characterization both of technical and plasma physical characteristics and of biological performance are very different and in most cases not comparable.

Therefore, basic criteria for the performance characterization of plasma devices should be helpful to establish this innovative technology for medical applications. Moreover,

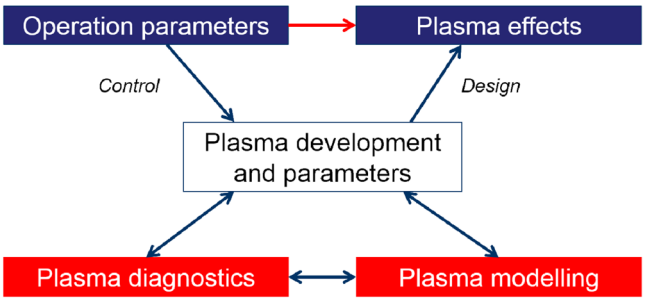

Figure 10. Basic elements of plasma device characterization.

such basic criteria are important to estimate the benefits and potential side effects of different CAP sources and will help to assess its biological performance and its potential for medical application at an early stage of development based on reproducible and more comparable tests. Additionally, such information will enhance the safety for investigators, patients, and therapists.

A useful possibility to define generally accepted basic criteria for processes or devices are national and international standards. Such standardization processes must be consensus-based and developed with the participation of all stakeholders and for the benefit of the society as a whole. To realize a fast transfer of knowledge and technology the special standardization tool of a specification, in Germany called DIN SPEC can be used. For such a DIN SPEC, informal or consortia standardization may be developed by a selected consortium. Such a specification is publicly available and can act as the basis for a further national, European or international standardization process.

In 2014, German DIN SPEC 'General requirements for plasma sources in medicine' was published (DIN SPEC 91315; Mann *et al* 2016). This developable specification describes obligatory basic criteria for the characterization of biocompatible cold atmospheric pressure plasma (CAP) sources which are supposed for medical applications. The DIN SPEC defines several physical (plasma temperature; thermal output; optical UV/VIS emission spectra; UV irradiance 200–400 nm; formation of toxic gases; leakage current) and biomedical criteria (chemical analysis of pH as well

H_2O_2, NO_2^-, and NO_3^- concentration in aqueous liquid; antimicrobial activity using five defined microorganisms; influence on human cell vitality–cytotoxicity using mandatory cell lines) to be tested. To estimate these parameters, simple assays are proposed which are easy to adapt, if common laboratory equipment is available.

The aim of a plasma device characterization according to DIN SPEC 91315 is not a comprehensive characterization of cold atmospheric plasma sources intended for biomedical application. However, it is suggested to get a primary set of basic data to describe basic performance characteristics of cold atmospheric plasmas in a standardized and better comparable manner. It is self-evident that dependent on the specific application additional specific tests have to be performed to characterize further performance. If necessary, such further tests can be also stipulated in standards.

Eventually, all these efforts to get a better comparability of different plasma devices with respect to its biological performance have to flow into the definition of a continuously available parameter or set of parameters to control and monitor plasma performance during application. To find such a device-independent possibility of plasma performance monitoring based on plasma characterization in the best case is possibly the main challenge for plasma physics and technology on its way to routine application in medicine.

5.3. Further basic research on mechanisms of plasma-cell and plasma-tissue interaction

To meet this challenge as well as to strengthen the scientific basis of plasma medicine, basic research has to be continued. Identification of plasma components responsible for specific biological effects is necessary both to come closer to a procedure of plasma performance monitoring as stated above, but also further optimize therapeutic plasma application and to enhance its safety. In this context it has to be investigated in detail how plasma effects in deeper layers of tissue or possibly distant to the treatment area, as it was repeatedly found in experimental treatment both of skin and tumors. Such effects could be based on diffusion of reactive oxygen and nitrogen species or its secondary products in the liquid phase of tissue but also by release of signalling substances by plasma-treated cells.

6. Summary

The use of cold atmospheric plasma opens up new therapeutic options in medicine.

Biological plasma effects are influenced but are also controllable by plasma parameters like treatment distance, (input) power, operation mode, working gas composition, treatment time, and environmental conditions. Biological and medically useful plasma effects are mainly caused by reactive oxygen and nitrogen species (ROS, RNS) which are generated locally and only for the required duration of the application on-site primarily by a physical process. These species are the same as occur in regular physiological and biochemical processes in the body but cannot supported adequately by drugs etc. because of limited stability. Because of its localized and short-term generation by local plasma treatment biologically active plasma components can be detoxified by processes of regular cell metabolism. As it was demonstrated by several *in vitro* studies and confirmed by first clinical experience, the risk of plasma application is low, assessable and manageable if the certified devices are careful operated according to the manufacturer's instructions. However, further basic as well as clinical research it necessary focused above all on long-term effects of plasma application.

The main clinical application field of cold atmospheric plasma is wound healing, yet. Further application fields in dentistry and oncology are foreseeable.

Plasma medicine is now as before at a state of introduction where a lot of further research and development is necessary. However, a considerable economic impact can be estimated, which is reflected by a growing economic interest and awareness.

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