BIOMOLECULES & THERAPEUTICS

Invited Review
Biomol Ther 22(6), 477-490 (2014)

Non-Thermal Atmospheric-Pressure Plasma Possible Application in Wound Healing

Beate Haertel[1], Thomas von Woedtke[2], Klaus-Dieter Weltmann[2] and Ulrike Lindequist[1,*]

[1]Department of Pharmaceutical Biology, Institute of Pharmacy, Ernst-Moritz-Arndt University of Greifswald, D17489 Greifswald, Germany, [2]Leibniz Institute of Plasma Science and Technology Greifswald e.V (INP), Felix-Hausdorff Str. 2, 17489 Greifswald, Germany

Abstract

Non-thermal atmospheric-pressure plasma, also named cold plasma, is defined as a partly ionized gas. Therefore, it cannot be equated with plasma from blood; it is not biological in nature. Non-thermal atmospheric-pressure plasma is a new innovative approach in medicine not only for the treatment of wounds, but with a wide-range of other applications, as e.g. topical treatment of other skin diseases with microbial involvement or treatment of cancer diseases. This review emphasizes plasma effects on wound healing. Non-thermal atmospheric-pressure plasma can support wound healing by its antiseptic effects, by stimulation of proliferation and migration of wound relating skin cells, by activation or inhibition of integrin receptors on the cell surface or by its pro-angiogenic effect. We summarize the effects of plasma on eukaryotic cells, especially on keratinocytes in terms of viability, proliferation, DNA, adhesion molecules and angiogenesis together with the role of reactive oxygen species and other components of plasma. The outcome of first clinical trials regarding wound healing is pointed out.

Key Words: Angiogenesis, Cell surface molecules, Cell viability, Non-thermal atmospheric-pressure plasma, Plasma-cell interaction, Reactive oxygen species, Wound healing

INTRODUCTION`

The number of patients with chronic infected wounds has been reported to increase constantly (Strausberg *et al*., 2007; Werdin *et al*., 2009; Günther and Machens 2014). In Germany, about 4.5 to 5 million people are concerned from chronic, non-healing wounds (Werdin *et al*., 2009). Many of them with vascular disease or diabetes suffer from chronic venous leg or foot ulcers due to a lack of proper wound healing. In consequence, they lose functional ability leading to a poor quality of life and to long-term hospitalization. Chronic wounds are not only a medical problem but also psychologically relevant. Conventional treatments are time consuming, and therefore very expensive, treatment costs in Germany are more than 5 billion € each year (Werdin *et al*., 2009).

Chronic inflammation with persistence of various bacteria including biofilm formation is a hallmark of the non-healing wounds. Bacterial concentrations exceeding 10^5 or 10^6 bacteria colony-forming units per gram of tissue have been shown to impair wound healing. In the majority of cases *Staphylococcus aureus* was identified in chronic wounds which caused together with the methicillin-resistant *Staphylococcus aureus* (MRSA) 20% to 50% of cases (Werdin *et al*., 2009). In Korea, the prevalence of community-associated MRSA infection is still low (Park *et al*., 2009); however, the prevalence of those MRSA strains in healthcare settings is increasing and currently accounts for up to 70% in most tertiary care hospitals (Kim *et al*., 2003a; Cha *et al*., 2005, 2010). MRSA infections contribute significantly to patient morbidity and mortality.

The initial step in the management of any chronic wound is cleaning them to eliminate excessive bacterial burden and necrotic tissue. Antimicrobial strategies are then used to remove or kill bacteria together with stimulation of patient's general health or the wound's physical environment (Daeschlein, 2013; Kramer *et al*., 2013). In chronic wound care antiseptics are effective and well tolerated. They play an important role in the treatment of wound infection; however, especially in treating infections by multidrug-resistant strains such as MRSA they have limitations. New concepts and strategies controlling wound inflammation and thus improving chronic wound care are strongly needed. One of these promising strategies is the application of physical non-thermal atmospheric-pressure

Open Access http://dx.doi.org/10.4062/biomolther.2014.105

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Received Sep 24, 2014 **Revised** Nov 10, 2014 **Accepted** Nov 10, 2014
Published online Nov 30, 2014

***Corresponding Author**
E-mail: lindequi@uni-greifswald.de
Tel: +49(0)3834-864868, Fax: +49(0)3834-864885

Copyright © 2014 The Korean Society of Applied Pharmacology

plasma (Lloyd *et al*., 2010).

HOW IS NON-THERMAL ATMOSPHERIC-PRESSURE PLASMA DEFINED?

Physical plasma has been considered as the fourth state of matter and is defined as a completely or partly ionized gas. Irvine Langmuir (1928) was the first who named ionized gas "plasma". In plasmas electrons, positive and negative ions, neutral atoms, and neutral or charged molecules can be identified. It is further characterized by its temperature, different types of radiation (e.g. UVB), and by electric fields (Fig. 1). Plasmas can be seen in daily life, e.g. as lightning in thunderstorms, northern lights, neon lights or plasma displays.

Plasmas can be "thermal/hot" and "non-thermal/cold". Thermal plasma is nearly fully ionized while non-thermal plasma is only partly ionized. Generating plasma artificially, it can be ignited at low or atmospheric pressure by adding energy to a gas, e.g. air, argon or helium. In a variety of different fields plasmas are applied. Plasma applications are found in technology and industry, e.g. in vehicle construction or metallurgy (von Woedtke *et al*., 2013).

The generation of plasma at atmospheric pressure with temperatures of about 30 to 40°C was the basis for treating living cells, tissues and other heat sensitive material. A new field, "Plasma Medicine", combining plasma physics with life science and medicine developed rapidly (von Woedtke *et al*., 2014). New plasma sources and devices were introduced for different applications.

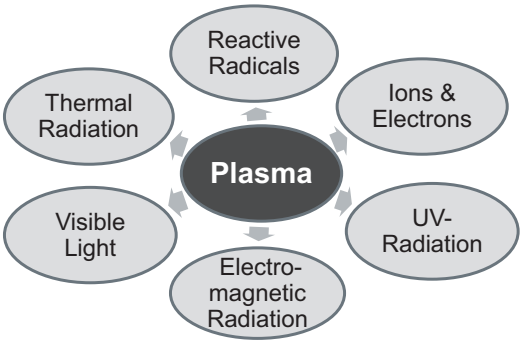

Fig. 1. Composition of non-thermal atmospheric-pressure plasmas.

PLASMA SOURCES FOR CELL AND TISSUE RESEARCH

At least three different principles of generating non-thermal plasmas at atmospheric pressure have been developed for biomedical applications (Weltmann *et al*., 2008; Hähnel *et al*., 2010; Ehlbeck *et al*., 2011; Wu *et al*., 2011; Bussiahn *et al*. 2013):

1. Plasma Jets
2. Corona discharge plasma sources
3. Dielectric barrier discharge (DBD) plasma sources

Our group has been working with experimental plasma sources belonging to two of these principles, the plasma jet kINPen 09 (principle 1; Fig. 2A), surface and volume barrier discharge (DBD) plasma sources (principle 3; Fig. 2B, C). All these plasma sources were developed at the Leibniz Institute for Plasma Science and Technology Greifswald e.V. (INP). Argon (kINPen 09, surface DBD, volume DBD), argon-oxygen mixtures (kINPen 09) or ambient air (surface DBD) were used as operating gas. Technical data of these plasma sources

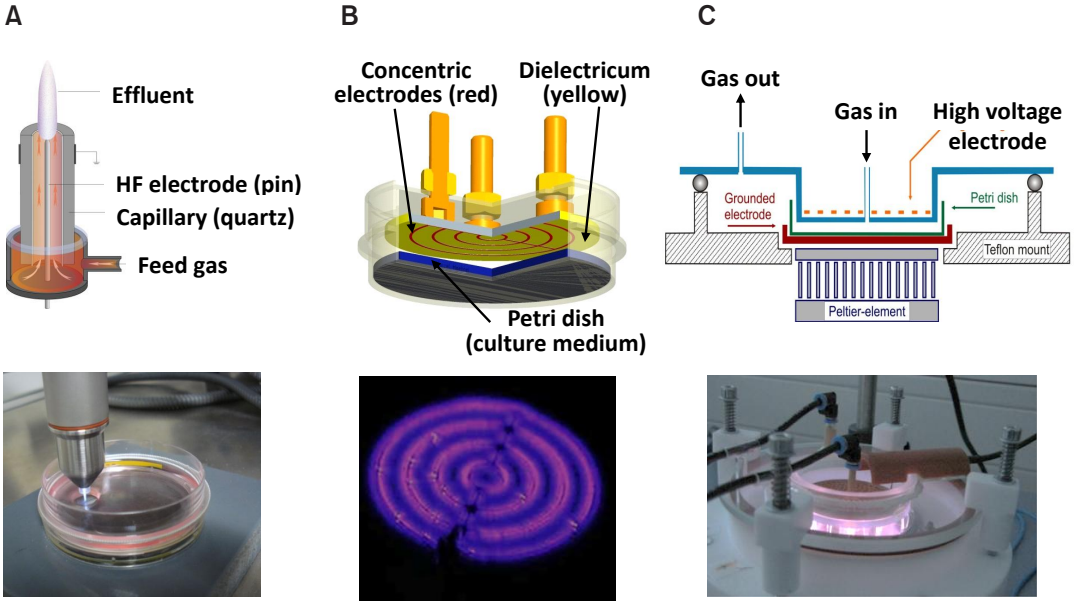

Fig. 2. Scheme of plasma sources used and the burning plasmas. (A) Plasma jet kINPen 09; (B) Surface DBD and (C) Volume DBD. All plasma sources were developed and built in the Leibniz Institute for Plasma Science and Technology (INP) in Greifswald, Germany.

Table 1. Technical data of the different plasma sources

Parameter	kINPen	Surface DBD	Surface DBD	Volume DBD
Process Gas	Argon	Ambient Air	Argon	Argon
Voltage	2- 6 kV	10 kV	3.5 kV	9-10 kV
Applied Frequency	1.1 MHz	20 kHz	21 kHz	33 kHz
Plasma on/off-time	---	0.413/1.223 s	0.413/1.223	---
Gas flow (Argon)	3.8 sl/min	---	0,5 sl/min	0.5 sl/min
Energy per min	< 60 J	18 J	8.25 J	360 J

are listed in Table 1. Energy output as sign for the power of a plasma source is lowest for the surface DBD with argon as process gas and highest for the volume DBD. Energy output is directly associated with inducing lethal or non-lethal effects on cells or microorganisms.

NON-THERMAL ATMOSPHERIC-PRESSURE PLASMA AND MICROORGANISMS

Non-thermal atmospheric-pressure plasma was found to inactivate very effectively different microorganisms (Hong *et al*., 2009; Hähnel *et al*., 2010; Kim *et al*., 2011a; Zimermann *et al*., 2011; Matthes *et al*., 2012; Daeschlein *et al*. 2012a; Li *et al*., 2013) and is able to remove biofilms (Joshi *et al*., 2010; Alkawareek *et al*., 2012; Fricke *et al*., 2012; Julak and Scholtz, 2013; Matthes *et al*., 2013). Even multidrug resistant skin and wound pathogens are susceptible to Non-thermal atmospheric-pressure plasma (Maisch *et al*., 2012; Daeschlein *et al*., 2014). Complete inactivation of various bacteria including the methicillin-resistant *Staphylococcus aureus* (MRSA) was reported by Alkawareek *et al*. (2014). All this led to the hypothesis that plasma might be an alternative solution for antiseptic treatment of chronic infected wounds (Kramer *et al*., 2013) or disinfection of surgical instruments or catheters (Polak *et al*., 2012; Robert *et al*., 2013; Sung *et al*., 2013).

Indeed, in terms of wound healing studies in experimental animals (Ermolaeva *et al*., 2011; Nastuta *et al*., 2011; Yu *et al*., 2011; García-Alcantara *et al*., 2013; Nasruddin *et al*., 2014) and humans (Isbary *et al*., 2012, 2013a; Heinlin *et al*., 2013b; Brehmer *et al*., 2014) demonstrated first positive effects. Recently, also other skin diseases came into focus for treatment with plasma, e.g. Morbus Hailey-Hailey (Isbary *et al*., 2011), pruritus (Heinlin *et al*., 2013a), atopic eczema (Emmert *et al*., 2013), psoriasis (Klebes *et al*., 2014).

The mechanisms by which plasma exerts its promising wound healing effects are still under investigation. Additionally to antibacterial effects plasma has also consequences for all other cells important for closing a wound. Here, we will review some effects of plasma, which are important regarding wound healing.

GENERAL EFFECTS OF NON-THERMAL ATMOSPHERIC-PRESSURE PLASMA ON WOUND RELATING SKIN CELLS

Effects of plasma were extensively investigated in vitro by using different types of cells in monolayer. Wound relating cells are keratinocytes, fibroblasts, epithelial and endothelial cells, but also inflammatory cells, especially in terms of chronic infected wounds. Studies were either done with cell lines or primary cells. The Greifswald group mainly deals with effects of plasma on keratinocytes (Haertel *et al*., 2011, 2012a, 2013a, 2013b; Blackert *et al*., 2013; Schmidt *et al*., 2013a, 2013b; Straβenburg *et al*., 2013; Straβenburg, 2014; Wende *et al*., 2014), namely the HaCaT cell line (Boukamp *et al*., 1988). Other groups focus either on epithelial cells (Kieft *et al*., 2004; Kalghatgi *et al*., 2011a, 2011b; Hoentsch *et al*., 2012), endothelial cells (Kalghatgi *et al*., 2010), ocular keratocytes (Brun *et al*., 2012), fibroblasts (Shashurin *et al*., 2010) or immune cells (Shi *et al*., 2008; Haertel *et al*., 2012b; Bekeschus *et al*., 2013a, 2013b, 2014; Bundscherer *et al*., 2013a, 2013b). For plasma treatment the different groups used various plasma sources. Basically, two principally different plasma sources were utilized: plasma jets and dielectric barrier discharge plasma sources. Up to now no general standardization of the different plasma sources with regard to technical data, quantification of generated free radicals or emission of radiation exists, which is, however, strongly demanded. Only in Germany a first "General requirements for plasma sources in medicine" is just published (DIN SPEC 91315, 2014), which were presented at the 5th International Conference on Plasma Medicine (ICPM5) by Mann *et al*. (2014). In that, simple and generally applicable biological (inactivation of microorganisms, cytotoxicity and detection of chemical species in liquids) and physical test methods (temperature, thermal capacity, optical emission spectrometry, UV-irradiation, gas emission, and leakage current) are proposed. These are basic criteria, which should be helpful to identify plasma sources for potential therapeutic applications. By using such standards plasma sources will achieve higher acceptance for dermatological and other medical applications. Taking all this into account, it is very difficult to compare the results of different laboratories published till now in terms of plasma treatment times/plasma doses which induce stimulating or lethal effects on cells or tissues. However, despite the use of different cell types or different plasma sources the following general plasma-treatment-time-dependent/plasma-dose-dependent effects were observed in all studies:

- Plasma membrane alteration,
- Induction of intracellular reactive oxygen radicals,
- Mitochondrial damage
- Induction of apoptosis and necrosis with decrease of cell viability and cell death,
- Increase or decrease of cell proliferation
- Increase or decrease of cell migration and
- DNA breakdown with cell cycle arrest.

All these effects are not only dependent on plasma treat-

Table 2. Relation between plasma sources, treatment regimens and treatment times which cause a reduction of viability of HaCaT keratinocytes of about 50% 24h after plasma exposure (experiments in RPMI 1640)

	kINPen 09 Argon	Surface-DBD Air	Surface-DBD Argon	Volume-DBD Argon
Direct Plasma Treatment				
Cells in Suspension	10 s	< 1 min	> 5 min	Ø
Adherent Cells	Ø	5 min	10 min	10 s
Indirect Plasma Treatment				
Cells in Suspension	Ø	< 1 min	< 5 min	Ø
Adherent Cells	1 min	Ø	Ø	10 s
Direct Plasma Treatment with medium exchange				
Cells in Suspension	Ø	1 min	Ø	Ø
Adherent Cells	Ø	> 20 min	Ø	> 1 min

ment time, but also on the process gas (ambient air, argon, helium), the treatment regimen (direct, indirect), the time of investigation after plasma exposure, the cell type and whether the cells were treated in suspension (immune cells) or as adherent cell monolayer (e.g. keratinocytes, fibroblasts).

It is very important to distinguish between plasma-induced lethal and plasma-induced stimulating effects on cells. The following statement is generally accepted:

- Short plasma treatment times/low plasma doses have stimulating effects (increase of proliferation and migration, induction of DNA repair) and
- Long plasma treatment times/high plasma doses induce lethal effects (cell death by apoptosis, stop of proliferation, DNA damage, cell cycle arrest).

The first reaction pattern is strongly demanded for improving wound healing, the latter properties can be used for treating cancer cells.

In the following sections of "GENERAL EFFECTS OF NON-THERMAL ATMOSPHERIC-PRESSURE PLASMA ON WOUND RELATING SKIN CELLS" we will describe effects of plasma on viability, apoptosis and proliferation, on DNA and on the role of reactive radicals.

Viability, apoptosis and proliferation

As already mentioned above, despite different physical parameters general effects on cell viability of plasma-treated cells are very similar. Determination of viability gives first information about the power of a given plasma treatment. Thinking about wound healing, microorganisms should be killed without harming keratinocytes or fibroblasts. For this reason HaCaT cells were treated with a broad range of plasma intensity/plasma treatment times, ranging from short to longer plasma exposure, to find plasma treatment times which do not induce lethal effects on keratinocytes (Haertel *et al*., 2011, 2012a, 2013a, 2013b; Blackert *et al*., 2013; Straβenburg *et al*., 2013; Straβenburg, 2014; Wende *et al*., 2014).

Comparing the plasma sources and treatment regimen main differences can be identified in the treatment time necessary to induce 50% cell death (Table 2). Treating cells directly with plasma, all the plasma components shown in Fig. 1 are relevant for the subsequent effects on the cells. In contrast, if cells are only exposed to plasma-treated medium (=indirect treatment), any effects on cells due to the different kinds of radiation are excluded. Similar results on viability after plasma treatment have also been reported by others for different other cell types, as e.g. immune cells (Shi *et al*., 2008; Haertel *et al*., 2012b; Bundscherer *et al*., 2013b; Bekeschus *et al*., 2103b), epithelial cells (Hoentsch *et al*., 2012, 2014; Kalghatgi *et al*., 2011a, 2011b, 2012), endothelial cells (Kalghatgi *et al*., 2010), fibroblasts (Lopes *et al*., 2013).

The working gas alone, argon or helium often used by others (Kieft *et al*., 2004; Shashurin *et al*., 2010; Brun *et al*., 2012), and short exposure times of cells to plasma were without any influence on cell viability. For any treatment regimen applied to the cells we observed that effects on cell viability were treatment-time-dependent. Direct and indirect plasma treatment caused very similar effects (Haertel *et al*., 2012a), thereby, major effects of any radiation emitted by plasma, e.g. UV radiation can be excluded. Viability is much improved if the medium is changed immediately after plasma treatment (Haertel *et al*., 2012a; Blackert *et al*., 2013).

An important factor for cell viability is the surrounding medium in which the cells are treated and cultured further. HaCaT cell number decreased in RPMI 1640 medium much more than in IMDM (Wende *et al*., 2014).The reason for this difference is the diverse composition of the culture media, which will be discussed later together with reactive oxygen species.

Mechanisms of reduced/enhanced cell viability can be reduction/promotion of cell proliferation or induction/prevention of apoptosis and/or necrosis. Indeed, by using the kINPen 09 or the surface DBD with ambient air reduction of HaCaT cell proliferation was detected, which correlated well with decrease of viability (Straβenburg, 2014; Wende *et al*., 2014). Otherwise, HaCaT cells on plasma-modified collagen films showed an increased proliferation (Garcia *et al*., 2010). Endothelial cell or fibroblast cell proliferation is enhanced by non-thermal plasma through release of fibroblast growth factor-2 or -7 (Kalghatgi *et al*., 2010; Ngo *et al*., 2014). Non-thermal plasma can induce apoptosis (Kim *et al*., 2011b; Haertel *et al*., 2012a, 2013b; Blackert *et al*., 2013; Duval *et al*., 2013; Wende *et al*., 2014). However, plasma induction of apoptosis measured by using Annexin V and propidium iodide (PI) was not observed after short plasma treatments. After longer plasma treatments apoptosis in HaCaT cells was still seen 24 h after plasma treatment (Blackert *et al*., 2013; Haertel *et al*., 2013b; Wende *et al*., 2014). To see apoptotic processes investigations have to be done early after treatment (e.g. 30 min to 4 h after plasma treatment). This is underlined by induction of early apoptosis (Annexin V/PI) in rat primary immune cells that was highest 4h after direct treatment with surface DBD/air and

Table 3. Examples of possible effects of reactive oxygen and nitrogen radicals (for review see Dröge, 2002)

Positive effects	Negative effects as "oxidative stress"
Signal transduction (NO)	Cell wall damage (ROS)
Stimulation of angiogenesis (NO)	Oxidation of DNA and proteins (O)
Influence on immune cells	Oxidation of lipids in cell bilayers (HO)
Proliferation of keratinocytes	Influence on cell respiration (O_3)
Smooth muscle relaxation (NO)	
Control of ventilation	
Antimicrobial effects (H_2O_2)	

was found to be reduced after 24 h and 48 h (Haertel *et al*., 2012b). Duval *et al*. (2013) detected more Annexin V/PI positive cells in Jurkat cells (a T-cell line) 24 h after plasma treatment compared to 8 h post treatment. These opposed results might be explained by the use different plasma sources and different cells. Another possibility to analyze apoptosis is the disruption of active mitochondria as distinctive feature of early apoptosis including changes in the mitochondrial membrane potential (Mito-MP). These changes can be measured by a lipophilic, cationic dye (JC-1) (Salvioli *et al*., 1997). Plasma treatment of HaCaT cells with the kINPen 09 induced changes in the Mito-MP 60 min after treatment. Four and 24 h after treatment only weak changes were observed.

In conclusion, if the plasma dose applied to the cells is high enough, cell death as result of more than one process is induced. At least induction of apoptosis/necrosis and reduction of proliferation due to cell cycle arrest (see under "Influence on DNA") play significant roles.

Reactive oxygen and nitrogen species and induction of intracellular reactive oxygen species

As already mentioned plasma emits several kinds of radiation and is further characterized by reactive oxygen and nitrogen species (ROS and RNS, Schaper *et al*., 2009; Schmidt-Bleker *et al*., 2014; Oehmigen, 2014). Among them, e.g. ozone/O_3 (Reuter *et al*., 2012b), nitric oxide/NO (Pipa *et al*., 2012), atomic oxygen/O (Reuter *et al*., 2012a), and hydroxyl radical /·HO (Winter *et al*., 2014) were detected. These reactive species exert lots of effects on cells which can be positive or even negative (Table 3). After treatment of cells in culture medium reactive species are detectable in the gas phase over the cells as well as in the culture medium (Fig. 3). By using an argon plasma jet the effluent is surrounded by ambient air, thereby in the gas phase are not only argon atoms but also species built from ambient air. Furthermore, all species can also enter the cells possibly by diffusion or can induce new species within the cells (Fig. 3). These can be detected as intracellular ROS (iROS) by different fluorescent dyes (DAF-2: Arjunan *et al*., 2011a; H_2DCFDA: Brun *et al*., 2012; Haertel *et al*., 2012a; CM-H_2DCFDA: Haertel *et al*., 2013b; carboxy-H_2DCFDA: Leduc *et al*., 2010; Ma *et al*., 2014).

Ozone being a neutral oxygen species is known to inactivate microorganisms (e.g. bacteria, viruses, fungi, yeast and protozoa), to stimulate oxygen metabolism and to activate the immune system. Thereby, it is widely used not only in food industry but also in medicine (Kim *et al*., 2003b). Ozonized water is used e.g. in dental medicine. Furthermore, medical ozone is also applied in the treatment of various diseases as e.g. circulatory disorders, macular degeneration, viral diseases or rheumatism (for review see Bocci *et al*., 2009, Elvis and Ekta, 2011). For local application ozone seems to be useful in the treatment of infected wounds (Białoszewski and Kowalewski, 2003). However, ozone has also disadvantages, mainly due to its potential of oxidation, peroxidation or generation of free radicals. As a component of plasma ozone may contribute to the effects observed in vitro after plasma treatment. This question was addressed by Kalghatgi *et al*. (2012) and our group (Haertel *et al*., 2013b) by using either mammalian breast epithelial cells (MCF10A) or human keratinocytes (HaCaT) in culture. For that, first we measured the concentration of ozone accumulated in the gas phase over the cells in medium during a 300 s treatment cycle (energy input about 9 J/cm^2) with DBD/air plasma in a closed system. A concentration of about 100 ppm which is 1000 times higher than the maximum allowable concentration (MAC) was detected. The DBD plasma source used by Kalghatgi *et al*. (2012) caused ozone concentrations of 182 ppm (4.65 J/cm^2) and of 30 ppm (1.95 J/cm^2) within 15s. Exposure of HaCaT cells to 300s DBD/air plasma resulted in a decrease of viable cells to about 20% while 100 ppm ozone did not significantly reduce cell viability (Haertel *et al*., 2013b). A concentration of 1000 ppm led to a reduction of about 50% of cell viability. Viability of cells was not analyzed by Kalghatgi *et al*. (2012); however, they found no DNA damage by ozone compared to DBD treatment. Ozone itself does not play a role in mediating the observed effects of plasma on

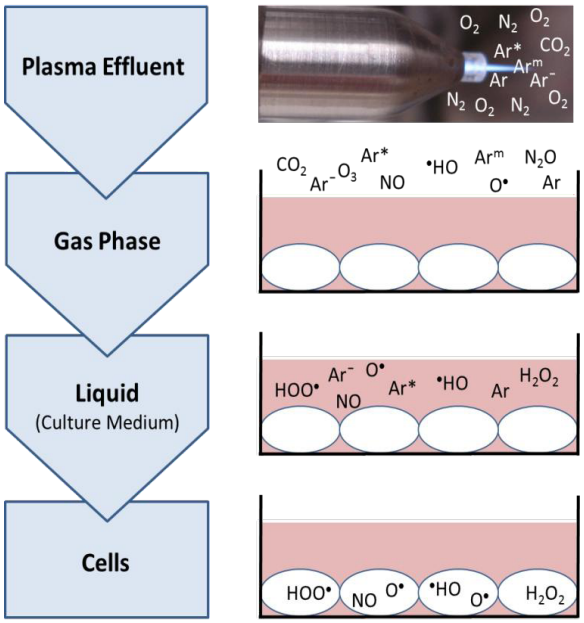

Fig. 3. Principle way of reactive oxygen and nitrogen species from an argon plasma jet over the gas phase and liquid into the treated cells. Since plasma jets are open systems the effluents are surrounded by ambient air with its gases N_2, O_2 and CO_2. Some species are exemplified shown. Species can enter the cell possibly by diffusion or can induce new species within the cells.

HaCaT keratinocytes or breast epithelial cells in culture.

To clarify whether other reactive oxygen species (ROS) have direct effects on viability, HaCaT cells were treated with 100 μM hydrogen peroxide (H_2O_2), a concentration which can be measured in the liquid after 300 s treatment with DBD/air. In liquids H_2O_2 can act with oxygen (O_2) to hydrogen peroxide radicals (HOO·), which then can form protons (H^+) and superoxide radicals (O2·). After exposure of HaCaT to H_2O_2 viability of cells was significantly decreased to 38%, which was very similar to that of plasma treated cells (Haertel *et al*., 2013b). These results clearly demonstrate that hydrogen peroxide itself or ROS built from it and not ozone is responsible for plasma-induced effects on cell viability. We further have to take into consideration that ROS can interact with components of the culture media and oxidize them.

To investigate whether or not these species penetrate from plasma over the liquid into the treated cells or whether intracellular ROS are induced, fluorescent dyes as already mentioned above were used to detect ROS intracellularly. Both mechanisms cannot be distinguished by measuring intracellular ROS by using CM-H_2DCFDA, but this method gives a general indication of the oxidation state of the cells following plasma treatment. By using this dye H_2O_2, peroxynitrite anion ($ONOO^-$), and hydroxyl radical (HO·), as well as alkylperoxyl and hydroxyl peroxyl radicals (ROO·, HOO·) can be detected. A plasma-treatment time dependent increase of iROS was found after exposure of HaCaT cells or human primary keratocytes to plasma (Brun *et al*., 2012; Haertel *et al*., 2013b; Straβenburg *et al*., 2013). Hydrogen peroxide (100 μM) and DBD/air treatment for 300 s caused similar results (Haertel *et al*., 2013b). Addition of a radical scavenger, e.g. N-acetyl cysteine (NAC), or a pre-treatment decreased the proportion of cells with enhanced iROS (Brun *et al*., 2012; Blackert *et al*., 2013) and completely blocked phosphorylation of H2AX after non-thermal plasma treatment of breast epithelial cells (Kalghatgi *et al*., 2012) underlying the crucial role of ROS for plasma-induced effects.

The effects of plasma on cells are significant dependent on the surrounding liquids. Various culture media differ in their composition markedly and thereby determine the extent of plasma effects considerably (Wende *et al*., 2014). They differ in their composition of sugars, amino acids, vitamins and buffer systems and they are characterized by different radical scavenging capacities. HEPES buffered media (e.g. IMDM) exerted highest scavenging activity (Wende *et al*., 2014). Therefore, it is not surprising that cells treated with plasma in IMDM survive better than after treatment in RPMI 1640 medium. HEPES in IMDM medium could scavenge ROS produced in the medium during plasma treatment. Hence, intracellular ROS concentration is reduced and oxidative effects within the cells, e.g. oxidation of DNA, are less. Addition of fetal calf serum (FCS) or antibiotics to culture media were found to be of minor importance (Wende *et al*., 2014).

Plasma and DNA

Since plasma components can enter the cells it is not surprising that also cell organelles including mitochondria or nuclei with its DNA are influenced. DNA damages can be base damages, deoxyribose modifications, single strand breaks (SSBs) or double strand breaks (DSBs) and DNA protein cross-links. Some of these damages can be repaired by the cells; however, DSBs are lethal to them. In this process reactive oxygen species play a central role and as we have demonstrated, ROS are detectable within the cells after plasma treatment. Hence, if the oxidative stress is high enough all four DNA bases can be oxidized by ROS (e.g. 8-hydroxy-2'-deoxyguanosine or N6-etheno-2'-deoxyadenosine) (Goetz and Luch, 2008). Different methods are used to recognize and detect changes in the DNA. First of all, the Comet assay as single cell gel electrophoresis detects single strand breaks (Singh *et al*., 1988). By using the neutral version of this test single but also double strand breaks are detectable. If double strand breaks occurred, this is followed by phosphorylation of the histone H2AX. This newly phosphorylated protein, γ-H2AX, is thereby a novel biomarker for DNA double-strand breaks (Kuo and Yang, 2008).

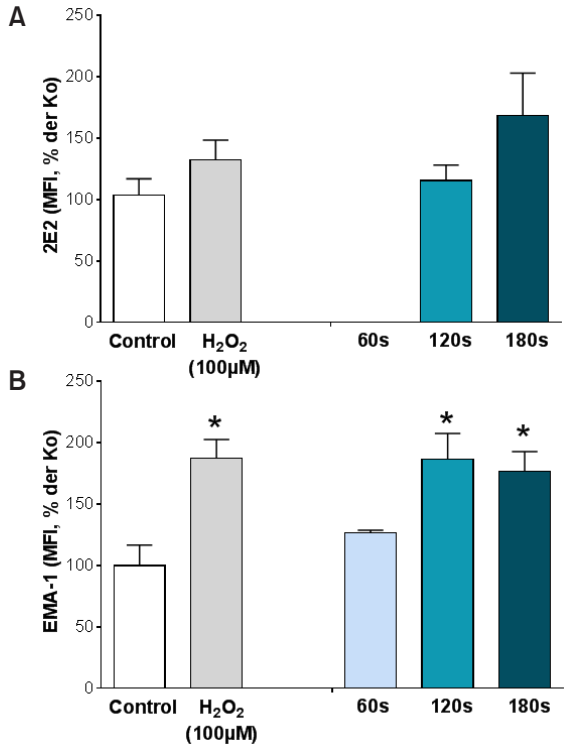

Fig. 4. Detection of oxidized DNA bases, namely (A) 8-hydroxy-2'-deoxyguanosine (8-OHdG) and (B) N6-etheno-2-deoxyadenosine after treatment of HaCaT keratinocytes with the kINPen 09 or hydrogen peroxide (100 μM). 24 h after exposure of cells to plasma they were fixed and stained with the antibodies 2E2 for 8-OHdG for and EMA-1 for N6-etheno-2'-desoxyadenosine. Binding of antibodies was detected by using flow cytometry. Mean fluorescence intensities (MFI) are expressed as percentage of that of untreated control cells. Mean ± SEM, *p<0.05 vs. untreated control cells.

There are several groups in the plasma community who detected DNA damages after plasma treatment by using different methods (γ-H2AX: Kalghatgi *et al*., 2011a, 2011b; 8-hydroxy-2'-deoxyguanosine (8-OHdG): Brun *et al*., 2012; Comet Assay: Blackert *et al*., 2013; Steinbeck *et al*., 2013; Morales-Ramirez *et al*., 2013; Straβenburg *et al*., 2013; Straβenburg, 2014; Wende *et al*., 2014). In our group the Comet Assay and detection of changed DNA bases, namely guanine to 8-OHdG and N6-etheno-2-deoxyadenosine, were used to define DNA damages (Blackert *et al*., 2013; Wende *et al*., 2014; Stra-

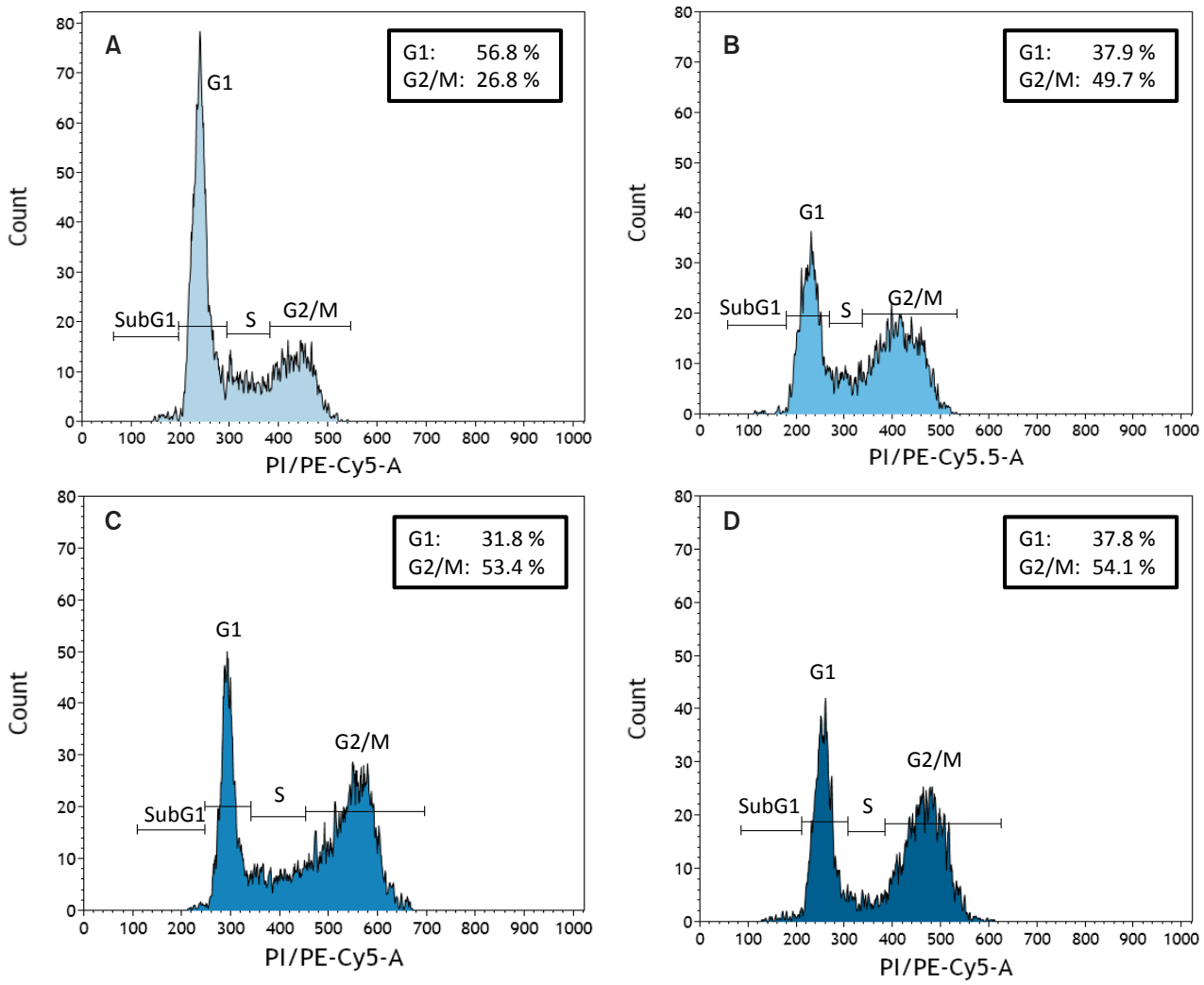

Fig. 5. Cell cycle analysis of HaCaT keratinocytes which stayed either untreated (A) or were treated with the kINPen 09 for 60s (B), surface DBD for 120 s (C) or volume DBD for 20 s (D). Analysis was done 24 h after exposure to plasma. Representative histograms with indication of the percentage of cells detected in the G1 and G2/M phase are shown. Plasma treatment induced independent of the plasma source used a typical G2/M.

βenburg *et al*., 2013; Straβenburg, 2014;). By using different plasma sources, the kINPen 09 (Wende *et al*., 2014), surface-DBD (Blackert *et al*., 2013) and volume-DBD (Straβenburg *et al*., 2013) treatment time-dependent DNA changes in HaCaT cells were detected 1 h and 24 h after plasma exposure. After short treatment cycles the induced DNA changes observed after 1 h of plasma exposure were no longer detectable after 24 h. Under these conditions viability of treated cells was not significantly influenced, thereby; this is a clear indication for DNA repair. Preliminary results give advice for induction of repair mechanisms (Straβenburg, 2014). As a consequence of double strand breaks in epithelial cells a plasma dose- or rather a time-dependent increase of γ-H2AX was detected 1 h after treatment with DBD plasma, which was completely blocked by the intracellular ROS scavenger NAC (Kalghatgi *et al*., 2011b). Similar results were reported for osteoblast-like cells (Steinbeck *et al*., 2013). γ-H2AX was analyzed 1 h after plasma treatment and short-term treatment was found to be negative in inducing phosphorylation of H2AX.

DNA base changes were observed after exposing HaCaT cells to the plasma jet kINPen 09 (Fig. 4). Flow cytometry was used to detect binding of corresponding antibodies, EMA-1 for N6-etheno-2'-desoxyadenosine and 2E2 for 8-hydroxy-2'-deoxyguanosine. While N6-etheno-2'-desoxyadenosine was found to be significantly increased by hydrogen peroxide and kINPen 09 treatment for at least 120 s (Fig. 4A), 8-hydroxy-2'-deoxyguanosine was only slightly enhanced after hydrogen peroxide and 180 s kINPen 09 exposure (Fig. 4B). These different results might be due to the fact that 8-hydroxy-2'-deoxyguanosine is the result from oxidation, while the DNA adduct N6-etheno-2'-deoxyadenosine arises from reaction of DNA with lipid peroxidation products (Taghizadeh *et al*., 2008). Lipid peroxidation can be the result of plasma treatment due to ROS generation. Transient increased expression of 8-hydroxy-2'-deoxyguanosine was also seen by Brun *et al*. (2012) in ocular keratocytes after exposing them to plasma.

Cell cycle analyses after plasma treatment give additional indication for influences on DNA. In HaCaT keratinocytes a G2/M phase arrest was detected after treating the cells with plasma. All plasma sources used induced comparable effects, however, in dependence on the plasma source with different plasma treatment times (Blackert *et al*., 2013; Straβenburg *et al*., 2013; Straβenburg, 2014; Wende *et al*., 2014). Representative examples for a G2/M arrest of HaCaT keratinocytes are shown in Fig. 5. HaCaT cells remained untreated (Fig. 5A), were either treated with the kINPen 09 for 60s (Fig. 5B), S-DBD for 120 s (Fig. 5C) or V-DBD for 20 s (Fig. 5D). G2/M arrest seems to be a typical sign after a given plasma treatment, since also others observed such a phenomenon not only in keratinocytes (Volotskova *et al*., 2012a) but particularly in different types of cancer cells (Vandamme *et al*., 2012; Volotskova *et al*., 2012a; Arndt *et al*., 2013; Köritzer *et al*., 2013). A cell cycle arrest in the G2/M phase gives cells time for DNA repair. Oxidative DNA damages will be detected, deleted and replaced by the DNA base excision repair (BER) pathway. In preliminary studies with HaCaT keratinocytes we looked for two enzymes (Ogg1 and APE-1) belonging to this repair pathway. By using the western blot technique, Ogg1, responsible for excision of 8-hydroxy-2'-deoxyguanosine, was found to be enhanced after kINPen 09 treatment of HaCaT cells (Kurth, 2013). These results are underlined by Brun *et al*. (2012) who demonstrated an increase of Ogg1 2 to 24h after a 2 min plasma treatment. In contrast, the repair enzyme APE-1 (apurinic/apyrimidinic endonuclease-1) was found to be reduced in HaCaT cells after kINPen 09 treatment (Kurth, 2013). This enzyme is the major human repair enzyme for abasic sites and incises the phosphodiester backbone 5' to the lesion to initiate a cascade of events aimed at removing the AP moiety and maintaining genetic integrity (Hadi *et al*., 2000). Up to now it is not clear whether the repair mechanism stops after deleting of 8-hydroxy-2'-deoxyguanosine by Ogg1.

SPECIFIC EFFECTS OF NON-THERMAL ATMOSPHERIC-PRESSURE PLASMA ON WOUND RELATING SKIN CELLS

Plasma and angiogenesis

Angiogenesis is a physiological process not only in embryogenesis but also in wound healing. Especially in chronic infected wounds aberrant angiogenesis is evident. In addition, growth and spread of solid tumors is dependent on formation of new blood vessels, which should be inhibited for a successful treatment. For improving wound healing angiogenesis should be promoted. Formation of new blood vessels is stimulated by a lack of oxygen and different endogenous pro-angiogenic factors. Among them are not only growth factors (VEGF, EGF, FGF) and cytokines (e.g. IL-1, 2, 6, 8; TNF, TGF) but also ROS and NO. Since plasma generates different ROS and NO, it was hypothesized that plasma should be able to stimulate angiogenesis. There are different methods to demonstrate an influence on the angiogenic process. Established in vitro methods use endothelial cells to measure simply their proliferation, migration or their ability to form tubes. Indeed, non-thermal plasma increased endothelial cell proliferation either by release of fibroblast growth factor-2 release (FGF-2), which is a promoter of angiogenesis (Kalghatgi *et al*., 2010) or by production of NO (Arjunan and Clyne, 2011a). Enhanced tube formation by using primary porcine aortic endothelial cells was found by Arjunan *et al*. (2012), who reported that particularly hydroxyl radicals and hydrogen peroxide seem to be responsible for the observed effects (Arjunan and Clyne, 2011b).

In our group more complex models like the rat aortic ring assay (AOR assay) and the in-ovo chick embryo chorioallantoic membrane assay (CAM assay) were used to measure the influence of non-thermal atmospheric-pressure plasma on the formation of new microvessels (Haertel *et al*., 2014). Either Matrigel-embedded aortic rings from LEW.1W or WOKW rats or chick embryo chorioallantoic membranes were indirectly treated with the plasma jet kINPen 09. Surprisingly, angiogenic response to plasma was found to be differentially influenced, depending on the models used and on the rat strain in the AOR test. In the CAM assay we found stimulation of angiogenesis, which could be quantified by fractal dimension and vessel area (Haertel *et al*., 2014). This effect was comparable to that observed with VEGF, a growth factor which secretion is stimulated by plasma e.g. from keratinocytes (Barton, 2013). Sprouting of microvessels from rat aortic rings was dependent on the rat strain used and either inhibited (WOKW) or not influenced (LEW.1W). It is difficult to explain this result, however, for mice it has been reported that the genetic background plays an essential role for VEGF-stimulated vessel sprouting in the aortic ring assay (Zhu *et al*., 2003).

Besides growth factors, cytokines, ROS and NO angiogenesis is fundamentally influenced by adhesion molecules, especially by integrin expression on endothelial cells mediating cell-matrix interaction. Non-thermal plasma is known to modify integrins on fibroblasts, keratinocytes and immune cells and thereby possibly also on endothelial cells. Future work should concentrate on the influence of plasma on the different key players influencing angiogenesis.

Plasma and cell surface molecules

In wound healing, cell adhesion plays a critical role for proliferation of cells as fibroblasts, keratinocytes and endothelial cells and their migration into the wound area. Cell adhesion is mediated by specialized molecules located on the cell surface which can be divided into cell-cell and cell-matrix adhesion molecules. These molecules are responsible for cell adhesion or detachment, for cell migration, cell signaling, growth and differentiation (Lauffenburger and Horwitz, 1996) and should be influenced by plasma according to the requirements.

Cell detachment often observed after treating cells with plasma (Stoffels *et al*., 2003; Kieft *et al*., 2004; Haertel *et al*., 2011; Hoentsch *et al*., 2012, 2014) provides a potent indication for the role of cell adhesion molecules, especially cell-matrix molecules. Integrins are transmembrane adhesion receptors which consist of a α- and a β-subunit. They mediate binding of cells to components of the extracellular matrix (ECM) and thereby, they are also responsible for cell migration. The role of integrin expression on fibroblasts, epithelial cells and HaCaT keratinocytes was underlined by investigations of Shashurin *et al*. (2010), Volotskova *et al*. (2012b) and our group (Haertel *et al*. 2011, 2012a, 2013a, 2013b). Treatment of adherent fibroblasts with a plasma jet was found to reduce expression of integrin β1 and αv on the cell surface (Shashurin *et al*., 2010). It is concluded that this is the original cause for cell detachment and reduced cell migration which was observed under similar conditions. In contrast, β1 integrin intensity was

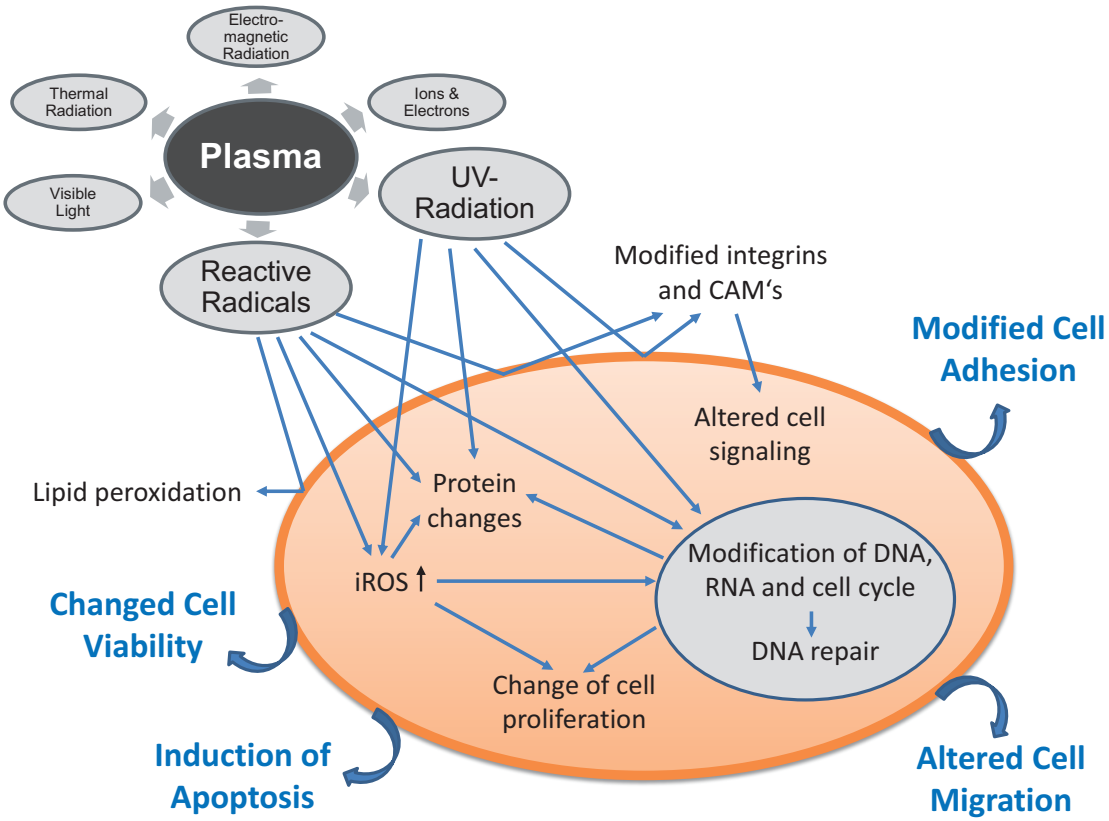

Fig. 6. Schematic summary of plasma effects on eukaryotic cells. Some interplay between plasma components e.g. reactive radicals or UV radiation and resulting effects are depicted. Effects on different levels of the cells were recognized.

reported to be increased after treating mouse fibroblasts by a plasma jet although migration rate of fibroblasts was found to be significantly reduced (Volotskova *et al*., 2012b). Analysis of αv integrin revealed no change in intensity. On the other hand, increased migration of a mouse fibroblast line after plasma treatment was reported by Ngo *et al*. (2014), however, without referring to adhesion molecules. Our group analyzed the expression of several integrins on the surface of HaCaT keratinocytes. For plasma effects it was very important whether the cells were treated as monolayer or as cell suspension. Cells in suspension are neither connected to each other by cell adhesion molecules (CAM's) as e.g. E-cadherin, nor to a matrix by integrins. In contrast, cells in monolayer are attached to a matrix and to surrounding cells. Therefore, they are not as sensitive to external influences as cells in suspension. Indeed, while cell number after treating HaCaT cells in suspension is already significantly reduced by a 20 s treatment cycle (Haertel *et al*., 2012a), monolayers can be exposed to surface DBD for 120 s before cell number is reduced (Haertel *et al*., 2013b). Integrin β1 was found to be up-regulated on HaCaT cells treated as suspension and as monolayer, however, treatment time to reach this result was longer for monolayers (120 s vs. 300 s). For regulation of integrin α2 an opposite behavior was detected after surface DBD treatment. While it was decreased on suspended cells, it increased on adherent cells. Stoffels *et al*. (2003) postulated that additionally to integrins, also cell-cell adhesion must be disturbed during cell detachment caused by plasma treatment. Indeed, we found a remarkable reduction of E-cadherin on HaCaT keratinocytes after kINPen 09 or surface DBD treatment (Haertel *et al*., 2011, 2012a). However, this result was only observed if the cells were treated in suspension. Treating a monolayer of HaCaT keratinocytes with plasma E-cadherin expression was not influenced (Haertel *et al*., 2013a, 2013b).

Detailed investigation of a greater panel of integrins after exposing HaCaT cells to surface DBD in monolayer revealed, in addition to an increase of α2 and β1 integrin, also an enhanced intensity for α5, α6 and β3 (Haertel *et al*., 2013b). The subunit α4 was never influenced and α3 and αv were slightly decreased (not significant). Regulation of β1 and αv integrin on HaCaT cells by plasma is in accordance with changes of those molecules on fibroblasts reported by Volotskova *et al*. (2012b). The observed effects of plasma on integrins were not mediated by ozone, but by reactive oxygen species as demonstrated for hydrogen peroxide (Haertel *et al*., 2013b). Only very high ozone concentrations (about 1800 ppm) increased integrin α2 comparable to surface DBD (300 s). As already mentioned during a 300 s treatment cycle with the surface DBD/air plasma about 100 ppm ozone was detected, which is 1000 times higher than the maximum allowable concentration (MAC).

The relevance of non-thermal atmospheric-pressure plasma for treating chronic infected wounds is not only given by its antimicrobial effects and stimulation of proliferation and migration of wound relating skin cells but also by its influence on cell adhesion receptors. Activation or inhibition of integrin recep-

tors by plasma may provide an excellent means of influencing wound healing. In particular, down-regulation of the integrin receptor $\alpha5\beta1$ in chronic wounds (Widgerow, 2013) could be enhanced by plasma. In contrast, $\alpha v\beta6$ is induced in chronic wounds and at least αv was decreased by plasma, however, not significantly. As demonstrated, plasma seems to be able to counteract the deleterious effects in chronic wounds in terms of integrin expression.

SUMMARY OF DETECTED IN VITRO EFFECTS OF NON-THERMAL ATMOSPHERIC-PRESSURE PLASMA

Figure 6 summarizes the effects of plasma on eukaryotic cells and tries to demonstrate some interplay between plasma components e.g. reactive radicals or UV radiation and resulting effects. Effects on different levels of the cells were recognized. First target is the cell membrane with its lipids and all embedded receptor proteins or enzymes. Lipid peroxidation and modification of cell adhesion molecules were observed resulting e.g. in an altered cell migration and cell signaling. Reactive molecules reach the cells possibly by diffusion, but they can also be induced within the cells and can thereby exert their effects e.g. on proteins. UV radiation and reactive radicals are further able to influence the DNA leading to a change of cell proliferation or induction of apoptosis. All these effects are dependent on the plasma dose/plasma treatment time and thereby both stimulating and deleterious effects are possible.

NON-THERMAL ATMOSPHERIC-PRESSURE PLASMA AND FIRST CLINICAL TRIALS REGARDING WOUND HEALING

Meanwhile, a good compatibility of plasma on skin has been reported. Plasma treatment of wounded pig skin, which closely resembles human skin, did not cause any toxic effects on the skin. Effective and fast blood coagulation was observed (Dobrynin *et al*., 2011). The authors concluded that plasma treatment is safe for living intact and wounded skin in plasma doses several times higher than required for inactivation of bacteria. Human skin physiology parameters were influenced by plasma, however, without damaging the skin or skin functions, indicating the safety of plasma under in vivo conditions (Fluhr *et al*., 2012). First clinical studies confirmed that plasma treatment was well tolerated, painless and without side effects (Isbary *et al*., 2010, 2012, 2013a; Daeschlein *et al*., 2012b; Emmert *et al*., 2013; Brehmer *et al*., 2014). However, future studies are needed to exclude long-term side effects. Regarding promotion of wound healing by plasma first clinical results are promising (Isbary *et al*., 2013b). Decrease of bacterial load in chronic wounds as presumption for an improved wound healing was shown in randomized controlled trials by using the atmospheric-pressure plasma jet MicroPlaSter plasma torch (Isbary *et al*., 2010, 2012). From a retrospective study of the same group it was concluded that wound healing may be accelerated by plasma, particularly for chronic venous ulcers (Isbary *et al*., 2013c). The plasma jet kINPen med® entails no risk for humans in terms of temperature increase, UV radiation or free radical formation and reduced bacterial load (Lademann *et al*., 2013). A different plasma device, the PlasmaDerm® VU-2010 device (CINOGY GmbH, Duderstadt, Germany) generating plasma by dielectric barrier discharge has also been shown to decrease bacterial load effectively in patients with chronic venous leg ulcers with more than 50% ulcer size reduction (Brehmer *et al*., 2014).

CONCLUSIONS

Taken together, non-thermal atmospheric-pressure plasma can support wound healing by its antiseptic effects, by stimulation of proliferation and migration of wound relating skin cells, by activation or inhibition of integrin receptors on the cell surface or by its pro-angiogenic effect. Non-thermal atmospheric-pressure plasma is a new innovative approach not only for the treatment of chronic wounds, but with a wide-range of other applications, as e.g. topical treatment of other skin diseases with microbial involvement or treatment of cancer diseases. Plasma parameters have to be defined for a safe application according to their needs. Norms for the technical devices to allow a standardized treatment of given diseases are very important and strongly needed. This is also the basis for comparison of the outcome of various trials conducted in different clinics. In future, effectivity of plasma treatment has to be demonstrated in controlled, randomized and greater clinical trials.

ACKNOWLEDGMENTS

The authors acknowledge Robert Koch, Christiane Meyer and Rüdiger Titze (Leibniz Institute for Plasma Sciences and Technology e.V.) for providing technical support. This study was realized within the joint research project “Campus PlasmaMed” supported by the German Federal Ministry of Education and Research (grant no. 13N9774 and 13N11182) as well as the project “Plasmamedical Research - New pharmaceutical and medical fields of application” funded by the Ministry of Education, Science and Culture of the State of Mecklenburg-Western Pomerania and the European Union, European Social Fund (grant number: AU 11 038; ESF/IV-BM-B35-0010/13).

None of the authors has to declare any conflict of interest including financial and other relationships.

REFERENCES

Alkawareek, M. Y., Algwari, Q. T., Laverty, G., Gorman, S. P., Graham, W. G., O'Connell, D. and Gilmore, B. F. (2012) Eradication of *Pseudomonas aeruginosa* biofilms by atmospheric pressure non-thermal plasma. *PLoS One* **7**, e44289.

Alkawareek, M. Y., Gorman, S. P., Graham, W. G. and Gilmore, B. F. (2014) Potential cellular targets and antibacterial efficacy of atmospheric pressure non-thermal plasma. *Int. J. Antimicrob. Agents* **43**, 154-60.

Arjunan, K. P. and Clyne, A. M. (2011a) A nitric oxide producing pin-to-hole spark discharge plasma enhances endothelial cell proliferation and migration. *Plasma Med.* **1**, 279-293.

Arjunan, K. P. and Clyne, A. M. (2011b) Hydroxyl radical and hydrogen peroxide are primarily responsible for dielectric barrier discharge plasma-induced angiogenesis. *Plasma Process. Polym.* **8**, 1154-1164.

Arjunan, K. P., Friedman G., Fridman A. and Clyne A. M. (2012) Non-thermal dielectric barrier discharge plasma induces angiogenesis through reactive oxygen species. *J. R. Soc. Interface* **9**, 147-157.

Arndt, S., Wacker, E., Li, Y. F., Shimizu,T., Thomas, H. M., Morfill, G. E., Karrer, S., Zimmermann, J.L. and Bosserhoff, A.K. (2013) Cold atmospheric plasma, a new strategy to induce senescence in melanoma cells. *Exp. Dermatol.* **22**, 284-289.

Barton A. (2013) Impact of non-thermal plasma on cell signaling in keratinocytes, Doctoral Thesis, Ernst-Moritz-Arndt-University of Greifswald, Germany.

Bekeschus, S., Kolata, J., Müller, A., Kramer, A., Weltmann, K. P., Bröker, B. and Masur, K. (2013a) Differential viability of eight human blood mononuclear cell subpopulations after plasma treatment. *Plasma Med.* **3**, 1-13.

Bekeschus, S., Kolata, J., Winterbourn, C., Kramer, A., Turner, R., Weltmann, K. D., Bröker, B. and Masur, K. (2014) Hydrogen peroxide: A central player in physical plasma-induced oxidative stress in human blood cells. *Free Radic. Res.* **48**, 542-549.

Bekeschus, S., Masur, K., Kolata, J., Wende, K., Schmidt, A., Bundscherer, L., Barton, A., Kramer, A., Bröker, B. and Weltmann, K. D. (2013b) Human mononuclear cell survival and proliferation is modulated by cold atmospheric plasma jet. *Plasma Process. Polym.* **10**, 706-713.

Białoszewski, D. and Kowalewski, M. (2003) Superficially, longer, intermittent ozone therapy in the treatment of the chronic, infected wounds. *Ortop. Traumatol. Rehabil.* **5**, 652-658.

Blackert, S., Haertel, B., Wende, K., von Woedtke, T. and Lindequist, U. (2013) Influence of non-thermal atmospheric pressure plasma on cellular structures and processes in human keratinocytes (HaCaT). *J. Dermatol. Sci.* **70**, 173-181.

Bocci, V., Borrelli, E., Travagli V. and Zanardi, I. (2009) The ozone paradox: ozone is a strong oxidant as well as a medical drug. *Med. Res. Rev.* **29**, 646-682.

Boukamp, P., Petrussevska, R. T., Breitkreutz, D., Hornung, J., Markham A. and Fusenig, N. E. (1988) Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J. Cell Biol.* **106**, 761-771.

Brehmer, F., Haenssle, H. A., Daeschlein, G., Ahmed, R., Pfeiffer, S., Görlitz, A., Simon, D., Schön, M.P., Wandke, D. and Emmert, S. (2014) Alleviation of chronic venous leg ulcers with a hand-held dielectric barrier discharge plasma generator (PlasmaDerm® VU-2010): results of a monocentric, two-armed, open, prospective, randomized and controlled trial (NCT01415622). *J. Eur. Acad. Dermatol. Venereol.*, in press, DOI: 10.1111/jdv.12490.

Brun, P., Vono, M., Venier, P., Tarricone, E., Deligianni, V., Martines, E., Zuin, M., Spagnolo, S., Cavazzana, R., Cardin, R., Castagliuolo, I., La Gloria Valerio, A. and Leonardi, A. (2012) Disinfection of ocular cells and tissues by atmospheric-pressure cold plasma. *PLoS One* **7**, e33245.

Bundscherer, L., Bekeschus, S., Tresp H., Hasse, S., Reuter, S., Weltmann, K. D., Lindequist, U. and Masur, K. (2013a) Viability of human blood leucocytes compared with their respective cell lines after plasma treatment. *Plasma Med.* **3**, 71-80.

Bundscherer, L., Wende, K., Ottmüller, K., Barton, A., Schmidt, A., Bekeschus, S., Hasse, S., Weltmann, K. D., Masur, K. and Lindequist, U. (2013b) Impact of non-thermal plasma treatment on MAPK signaling pathways of human immune cell lines. *Immunobiology* **218**, 1248-1255.

Bussiahn, R., Lembke, N., Gesche, R., von Woedtke, T. and Weltmann, K. D. (2013) Plasma sources for biomedical applications. *Hyg. Med*. **38**, 212-216.

Cha, H. Y., Kim, H. O., Jin, J. S. and Lee J. C. (2010) Emergence of vancomycin-intermediate *Staphylococcus aureus* from predominant methicillin-resistant *S. aureus* clones in a Korean hospital. *J. Microbiol.* **48**, 533-535.

Cha, H. Y., Moon, D. C., Choi, C. H., Oh, J. Y., Jeong, Y. S., Lee, Y. C., Seol, S. Y., Cho, D. T., Chang, H. H., Kim, S. W. and Lee J. C. (2005) Prevalence of the ST239 clone of methicillin-resistant *Staphylococcus aureus* and differences in antimicrobial susceptibilities of ST239 and ST5 clones identified in a Korean hospital. *J. Clin. Microbiol.* **43**, 3610-3614.

Daeschlein, G. (2013) Antimicrobial and antiseptic strategies in wound management. *Int. Wound J.* **10**(Suppl. 1), 9-14.

Daeschlein, G., Napp, M., von Podewils, S., Lutze, S., Emmert, S., Lange, A., Klare, I., Haase, H., Gümbel, D., von Woedtke, T. and Jünger, M. (2014) In vitro susceptibility of multidrug resistant skin and wound pathogens against low temperature atmospheric pressure plasma jet (APPJ) and dielectric barrier discharge plasma (DBD). *Plasma Process. Polym.* **11**, 175-183.

Daeschlein, G., Scholz, S., Ahmed, R., Majumdar, A., von Woedtke, T., Haase, H., Niggemeier, M., Kindel, E., Brandenburg, R., Weltmann, K. D. and Jünger, M. (2012b) Cold plasma is well-tolerated and does not disturb skin barrier or reduce skin moisture. *J. Dtsch. Dermatol. Ges.* **10**, 509-515.

Daeschlein, G., Scholz, S., Ahmed, R., von Woedtke, T., Haase, H., Niggemeier, M., Kindel, E., Brandenburg, R., Weltmann, K. D. and Jünger, M. (2012a) Skin decontamination by low-temperature atmospheric pressure plasma jet and dielectric barrier discharge plasma. *J. Hosp. Infect.* **81**, 177-183.

DIN SPEC 91315 (2014) "General requirements for plasma sources in medicine", DIN Deutsches Institut für Normung e.V., Beuth Verlag Berlin, June 2014. http://www.spec.din.de/cmd?level=tpl-art-detailansicht&committeeid=0&artid=203493369&languageid=en&bcrumblevel=2

Dobrynin, D., Wu, A., Kalghatgi, S., Park, S., Shainsky, N., Wasko, K., Dumani, E., Ownbey, R., Joshi, S., Sensing, R. and Brooks, A. D. (2011) Live pig skin tissue and wound toxicity of cold plasma treatment. *Plasma Med.* **1**, 93-108.

Dröge W. (2002) Free radicals in the physiological control of cell function. *Physiol. Rev.* **82**, 47-95.

Duval, A., Marinov, I., Bousquet, G., Gapihan, G., Starikovskaia, S. M., Rousseau, A. and Janin, A. (2013) Cell death induced on cell cultures and nude mouse skin by non-thermal, nanosecond-pulsed generated plasma. *PLoS One* **8**, e83001.

Ehlbeck, J., Schnabel, U., Polak, M., Winter, J., von Woedtke, T., Brandenburg, R., von dem Hagen, T. and Weltmann, K. D. (2011) Low temperature atmospheric pressure plasma sources for microbial decontamination. *J. Phys. D: Appl. Phys.* **44**, 013002.

Elvis, A. M. and Ekta, J. S. (2011) Ozone therapy: A clinical review. *J. Nat. Sci. Biol. Med.* **2**, 66-70.

Emmert, S., Brehmer, F., Hänβle, H., Helmke, A., Mertens, N., Ahmed, R., Simon, D., Wandke, D., Maus-Friedrichs, W., Daeschlein, G., Schön, M.P. and Viöl, W. (2013) Atmospheric pressure plasma in dermatology: Ulcus treatment and much more. *Clin. Plasma Med.* **1**, 24-29.

Ermolaeva, S. A., Varfolomeev, A. F., Chernukha, M. Y., Yurov, D. S., Vasiliev, M. M., Kaminskaya, A. A., Moisenovich, M. M., Romanova, J. M., Murashev, A. N., Selezneva, I. I., Shimizu, T., Sysolyatina, E. V., Shaginyan, I. A., Petrov, O. F., Mayevsky, E. I., Fortov, V. E., Morfill, G. E., Naroditsky, B. S. and Gintsburg, A. L. (2011) Bactericidal effects of non-thermal argon plasma in vitro, in biofilms and in the animal model of infected wounds. *J. Med. Microbiol.* **60**, 75-83.

Fluhr, J. W, Sassning, S., Lademann, O., Darvin, M. E., Schanzer, S., Kramer, A., Richter, H., Sterry, W. and Lademann, J. (2012) In vivo skin treatment with tissue-tolerable plasma influences skin physiology and antioxidant profile in human stratum corneum. *Exp. Dermatol.* **21**, 130-134.

Fricke, K., Koban, I., Tresp, H., Jablonowski, L., Schroder, K., Kramer, A., Weltmann, K. D., von Woedtke, T., and Kocher, T. (2012) Atmospheric pressure plasma: a high-performance tool for the efficient removal of biofilms. *PLoS One* **7**, e42539.

Garcia, J. L., Asadinezhad, A., Pacherník, J., Lehocký, M., Junkar, I., Humpolícek, P., Sáha, P. and Valásek, P. (2010) Cell proliferation of HaCaT keratinocytes on collagen films modified by argon plasma treatment. *Molecules* **15**, 2845-2856.

García-Alcantara, E., López-Callejas, R., Morales-Ramírez, P. R., Peña-Eguiluz, R., Fajardo-Muñoz, R., Mercado-Cabrera, A., Barocio, S. R., Valencia-Alvarado, R., Rodríguez-Méndez, B. G., Muñoz-Castro, A. E., de la Piedad-Beneitez, A. and Rojas-Olmedo, I. A. (2013) Accelerated mice skin acute wound healing in vivo by combined treatment of argon and helium plasma needle. *Arch. Med. Res.* **44**, 169-177.

Goetz, M. E. and Luch, A. (2008) Reactive species: a cell damaging rout assisting to chemical carcinogens. *Cancer Lett.* **266**, 73-83.

Günther, C.I. and Machens, H.G. (2014) Innovations in wound medicine. *Wound Med*. **4**, 9-12.

Hadi, M. Z., Coleman, M. A., Fidelis, K., Mohrenweiser, H. W. and Wil-

son, D. M. 3rd. (2000) Functional characterization of Ape1 variants identified in the human population. *Nucleic Acids Res.* **28**, 3871-3879.

Haertel, B., Eiden, K., Deuter, A., Wende, K., von Woedtke, T., Lindequist, U. (2014) Differential effect of non-thermal atmospheric-pressure plasma on angiogenesis. *Lett. Appl. NanoBioSci.* **3**, 159-166.

Haertel, B., Hähnel, M., Blackert, S., Wende, K., von Woedtke, T. and Lindequist, U. (2012a) Surface molecules on HaCaT keratinocytes after interaction with non-thermal atmospheric pressure plasma. *Cell Biol. Int.* **36**, 1217-1222.

Haertel, B., Straβenburg, S., Harms, M., Wende, K., Lindequist, U. and von Woedtke, T. (2013a) Biological effects of non-thermal atmospheric-pressure plasma on human HaCaT-keratinocytes. *Hyg. Med.* **38**, 198-205.

Haertel, B., Straβenburg, S., Oehmigen, K., Wende, K., von Woedtke, T. and Lindequist, U. (2013b) Differential influence of components resulting from atmospheric-pressure plasma on integrin expression of human HaCaT keratinocytes. *BioMed. Res. Int.* **2013**, 761451.

Haertel, B., Volkmann, F., von Woedtke, T. and Lindequist, U. (2012b) Differential sensitivity of lymphocyte subpopulations to non-thermal atmospheric-pressure plasma. *Immunobiology* **217**, 628-633.

Haertel, B., Wende, K., von Woedtke, T., Weltmann, K. D. and Lindequist, U. (2011) Non-thermal atmospheric-pressure plasma can influence cell adhesion molecules on HaCaT-keratinocytes. *Exp. Dermatol.* **20**, 282-284.

Hähnel, M., von Woedtke, T. and Weltmann, K. D. (2010). Influence of the air humidity on the reduction of bacillus spores in a defined environment at atmospheric pressure using a dielectric barrier surface discharge. *Plasma Process. Polym.* **7**, 244-249.

Heinlin, J., Isbary, G., Stolz, W., Zeman, F., Landthaler, M., Morfill, G., Shimizu, T., Zimmermann, J. L. and Karrer, S. (2013a) A randomized two-sided placebo-controlled study on the efficacy and safety of atmospheric non-thermal argon plasma for pruritus. *J. Eur. Acad. Dermatol. Venereol.* **27**, 324-331.

Heinlin, J., Zimmermann, J. L., Zeman, F., Bunk, W., Isbary, G., Landmthaler, M., Maisch, T., Monetti, R., Morfill, G., Shimizu, T., Steinbauer, J., Stolz, W., and Karrer, S. (2013b) Randomized placebo-controlled human pilot study of cold atmospheric argon plasma on skin graft donor sites. *Wound Repair Regen.* **21**, 800-807.

Hoentsch, M., von Woedtke, T., Weltmann, K. D. and Nebe, J. B. (2012) Time-dependent effects of low-temperature atmospheric-pressure argon plasma on epithelial cell attachment, viability and tight junction formation in vitro. *J. Phys. D: Appl. Phys.* **45**, 025206.

Hoentsch, M., Bussiahn, R., Rebl, H., Bergemann, C., Eggert, M., Frank, M., von Woedtke, T. and Nebe, J. B. (2014) Persistent Effectivity of gas plasmaa-treated, long time-stored liquid on epithelial cell adhesion capacity and membrane morphology. *PLoS One* **9**, e104559.

Hong, Y. F., Kang, J. G., Lee, H. Y., Uhm, H. S., Moon, E. and Park, Y. H. (2009) Sterilization effect of atmospheric plasma on *Escherichia coli* and *Bacillus subtilis* endospores. *Lett. Appl. Microbiol.* **48**, 33-37.

Isbary, G., Heinlin, J., Shimizu, T., Zimmermann, J. L., Morfill, G., Schmidt, H. U., Monetti, R., Steffes, B., Bunk, W., Li, Y., Klaempfl, T., Karrer, S., Landthaler, M. and Stolz, W. (2012) Successful and safe use of 2 min cold atmospheric argon plasma in chronic wounds: results of a randomized controlled trial. *Br. J. Dermatol.* **167**, 404-410.

Isbary, G., Köritzer, J., Mitra, A., Li, Y. F., Shimizu, T., Schroeder, J., Schlegel, J., Morfill, G. E., Stolz, W. and Zimmermann, J. L. (2013c) Ex vivo human skin experiments for the evaluation of safety of new cold atmospheric plasma devices. *Clin. Plasma Med.* **1**, 36-44.

Isbary, G., Morfill, G., Schmidt, H. U., Georgi, M., Ramrath, K., Heinlin, J., Karrer, S., Landthaler, M., Shimizu, T., Steffes, B., Bunk, W., Monetti, R., Zimmermann, J. L., Pompl, R. and Stolz, W. (2010) A first prospective randomized controlled trial to decrease bacterial load using cold atmospheric argon plasma on chronic wounds in patients. *Br. J. Dermatol.* **163**, 78-82.

Isbary, G., Morfill, G., Zimmermann, J., Shimizu, T. and Stolz, W. (2011) Cold atmospheric plasma: a successful treatment of lesions in Hailey-Hailey disease. *Arch. Dermatol.* **147**, 388-390.

Isbary, G., Stolz, W., Shimizu, T., Monetti, R., Bunk, W., Schmidt, H. U., Morfill, G. E., Klämpfl, T. G., Steffes, B., Thomas, H. M., Heinlin, J., Karrer, S., Landthaler, M. and Zimmermann, J. L. (2013a) Cold atmospheric argon plasma treatment may accelerate wound healing in chronic wounds: Results of an open retrospective randomized controlled study in vivo. *Clin. Plasma Med.* **1**, 25-30.

Isbary, G., Zimmermann, J. L, Shimizu, T., Li, Y. F., Morfill G., Thomas, H. M., Steffes, B., Heinlin, J., Karrer, S. and Stolz W. (2013b) Non-thermal plasma-More than five years of clinical experience. *Clin. Plasma Med.* **1**, 19-23.

Joshi, S. G., Paff, M., Friedman, G., Fridman, G., Fridman, A., and Brooks A. D. (2010) Control of methicillin-resistant *Staphylococcus aureus* in planktonic form and biofilms: A biocidal efficacy study of non-thermal dielectric-barrier discharge plasma. *Am. J. Infect. Control* **38**, 293-301.

Julak, J. and Scholtz, V. (2013) Decontamination of human skin by low-temperature plasma produced by cometary discharge. *Clin. Plasma Med.* **1**, 31-34.

Kalghatgi, S. and Azizkhan-Clifford, J. (2011a) DNA damage in mammalian cells by atmospheric pressure microsecond-pulsed dielectric barrier discharge plasma is not mediated via lipid peroxidation. *Plasma Med.* **1**, 167-177.

Kalghatgi, S., Fridman, A., Azizkhan-Clifford, J. and Friedman, G. (2012) DNA damage in mammalian cells by non-thermal atmospheric pressure microsecond pulsed dielectric barrier discharge plasma is not mediated by ozone. *Plasma Process. Polym.* **9**, 726-732.

Kalghatgi, S., Friedman, G., Fridman, A. and Clyne, A. M. (2010) Endothelial cell proliferation is enhanced by low dose non-thermal plasma through fibroblast growth factor-2 release. *Ann. Biomed. Eng.* **38**, 748-757.

Kalghatgi, S., Kelly, C., Cerchar, E., Torabi B., Alekseev, O., Fridman, A., Friedman, G. and Azizkhan-Clifford, J. (2011b) Effects of non-thermal plasma on mammalian cells. *PLoS One* **6**, e16270.

Kieft, I. E., Broers, J. L., Caubet-Hilloutou, V., Slaaf, D. W., Ramaekers, F. C. and Stoffels, E. (2004) Electric discharge plasmas influence attachment of cultured CHO K1 cells. *Bioelectromagnetics* **25**, 362-368.

Kim, H. B., Park, W. B., Lee, K. D., Choi, Y. J., Park, S. W., Oh, M., Kim, E. C. and Choe, K. W. (2003a) Nationwide surveillance for *Staphylococcus aureus* with reduced susceptibility to vancomycin in Korea. *J. Clin. Microbiol.* **41**, 2279-2281.

Kim, J. G., Yousef, A. E. and Khadre, M. A. (2003b) Ozone and its current and future application in the food industry. *Adv. Food Nutr. Res.* **45**, 167-218.

Kim, P. Y., Kim, Y. S., Koo, I. G., Jung, J. C., Kim, G. J., Choi, M. Y., Yu, Z. Q. and Collins, G. J. (2011a) Bacterial inactivation of wound infection in a human skin model by liquid-phase discharge plasma. *PLoS One* **6**, e24104.

Kim, W., Woo, K. C., Kim, G. C. and Kim, K. T. (2011b) Non-thermal-plasma-mediated animal cell death. *J. Phys. D: Appl. Phys.* **44**, 013001.

Klebes, M., Lademann, J., Philipp, S., Ulrich, C., Patzelt, A., Ulmer, M., Kluschke, F., Kramer, A., Weltmann, K. D., Sterry, W. and Lange-Asschenfeldt, B. (2014) Effects of tissue-tolerable plasma on psoriasis vulgaris treatment compared to conventional local treatment: A pilot study. *Clin. Plasma Med.,* in press, DOI: 10.1016/j.cpme.2013.11.002.

Köritzer, J., Boxhammer, V., Schäfer, A., Shimizu, T., Klämpfl, T. G., Li, Y. F., Welz, C., Schwenk-Zieger, S., Morfill, G. E., Zimmermann, J. L. and Schlegel, J. (2013) Restoration of sensitivity in chemoresistant glioma cells by cold atmospheric plasma. *PLoS One* **8**, e64498.

Kramer, A., Lademann, J., Bender, C., Sckell, A., Hartmann, B., Münch, S., Hinz, P., Ekkernkamp, A., Matthes, R., Koban, I., Partecke, I., Heidecke, C. D., Masur, K., Reuter, S., Weltmann, K. D., Koch, S. and Assadian, O. (2013) Suitability of tissue tolerable plasmas (TTP) for the management of chronic wounds. *Clin. Plasma Med.* **1**, 11-18.

Kuo, L. J. and Yang, L. X. (2008) γ-H2AX - A novel biomarker for DNA double-strand breaks. *In Vivo* **22**, 305-310.

Kurth, A. (2013) Einfluss von physikalischem Plasma auf in vitro kultivierte HaCaT-Zellen: Einfluss auf DNA und Reparaturmechanismen.

Diploma Thesis, Ernst-Moritz-Arndt-University Greifswald.

Lademann, J., Ulrich, C., Patzelt, A., Richter, H., Kluschke, F., Klebes, M., Lademann, O., Kramer, A., Weltmann, K. D. and Lange-Asschenfeldt, B. (2013) Risk assessment of the application of tissue-tolerable plasma on human skin. *Clin. Plasma Med.* **1**, 5-10.

Langmuir, I. (1928) Oscillations in ionized gases. *Proc. Nat. Acad. Sci. U.S.A.* **14**, 627-637.

Lauffenburger, D. A. and Horwitz, A. F. (1996) Cell migration: a physically integrated molecular process. *Cell* **84**, 359-369.

Leduc, M., Guay, D., Coulombe S. and Leask R. L. (2010) Effects of non-thermal plasmas on DNA and mammalian cells. *Plasma Process. Polym.* **7**, 899-909.

Li, Y. F., Taylor, D., Zimmermann, J. L., Bunk, W., Monetti, R., Isbary, G., Boxhammer, V., Schmidt, H. U., Shimizu, T., Thomas, H. M. and Morfill, G. E. (2013) In vivo skin treatment using two portable plasma devices: Comparison of a direct and an indirect cold atmospheric plasma treatment. *Clin. Plasma Med.* **1**, 35-39.

Lloyd, G, Friedman, G., Jafri, S., Schultz, G., Fridman, A., Harding, K. (2010) Gas plasma: medical uses and developments in wound care. *Plasma Process. Polym.* **7**, 194-211.

Lopes, B. B., Kraft, M. B. P. L., Rehder, J., Batista, F. R. X. and Puzzi, M. B. (2013) The interactions between non-thermal atmospheric pressure plasma and ex-vivo dermal fibroblasts. *Procedia Engin.* **59**, 92-100.

Ma Y, Ha, C. S., Hwang, S. W., Lee, H. J., Kim, G. C., Lee, K. W. and Song, S. (2014) Non-thermal atmospheric pressure plasma preferentially induces apoptosis in p53-mutated cancer cells by activating ros stress-response pathways. *PLoS One* **9**, e91947.

Maisch, T., Shimizu, T., Li, Y. F., Heinlin, J., Karrer, S., Morfill, G. and Zimmermann, J. L. (2012) Decolonisation of MRSA, *S. aureus* and *E. coli* by cold-atmospheric plasma using a porcine skin model in vitro. *PLoS One* **7**, e34610.

Mann, M., Tiede, R., Ahmed, R., Wandtke, D., Wurster, S., Weltmann, K. D., Deaschlein, G., Emmert, S. and von Woedtke, T. (2014) Standards in plasma medicine: development, contents and importance of the first German DIN specification. *ICPM5*, May 18-23, 2014, Nara, Japan.

Matthes, R., Bekeschus, S., Bender, C., Koban, I., Hübner, N. O. and Kramer A. (2012) Pilot-study on the influence of carrier gas and plasma application (open resp. delimited) modifications on physical plasma and its antimicrobial effect against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *GMS Krankenhaushyg. Interdiszipl*. **7**, 1-7.

Matthes, R., Bender, C., Schlüter, R., Koban, I., Bussiahn, R., Reuter, S., Lademann, J., Weltmann, K. D. and Kramer, A. (2013) Antimicrobial efficacy of two surface barrier discharges with air plasma against in vitro biofilms. *PLoS One* **8**, e70462.

Morales-Ramírez, P., Cruz-Vallejo, V., Peña-Eguiluz, R., López-Callejas, R., Rodríguez-Méndez, B.G., Valencia-Alvarado, R., Mercado-Cabrera, A. and Muñoz-Castro, A. E. (2013) Assessing cellular DNA damage from a helium plasma needle. *Radiat. Res.* **179**, 669-673.

Nasruddin, Nakajima, Y., Mukai, K., Rahayu, H. S. E., Nur, M., Ishijima, T., Enomoto, H., Uesugi, Y., Sugama, J. and Nakatani, T. (2014) Cold plasma on full-thickness cutaneous wound accelerates healing through promoting inflammation, re-epithelialization and wound contraction. *Clin. Plasma Med.*, in press, DOI: 10.1016/j.cpme.2014.01.001.

Nastuta, A. V., Topala, I., Grigoras, C., Pohoata, V. and Popa, G. (2011) Stimulation of wound healing by helium atmospheric pressure plasma treatment. *J. Phys. D: Appl. Phys.* **44**, 105204.

Ngo, M. H. T., Liao, J. D., Shao, P. L., Weng, C. C. and Chang, C. Y. (2014) Increased fibroblast cell proliferation and migration using atmospheric N_2/Ar micro-plasma for the stimulated release of fibroblast growth factor-7. *Plasma Process. Polym.* **11**, 80-88.

Oehmigen K. (2014) Plasma-Flüssigkeit-Wechselwirkungen. *Doctoral Thesis*, Ernst-Moritz-Arndt-University of Greifswald.

Park, S. H., Park, C., Yoo, J. H., Choi, S. M., Choi, J. H., Shin, H. H., Lee, D. G., Lee, S., Kim, J., Choi, S. E., Kwon, Y. M. and Shin, W. S. (2009) Emergence of community-associated methicillin-resistant *Staphylococcus aureus* strains as a cause of healthcare-associated bloodstream infections in Korea. *Infect. Control Hosp. Epidemiol.* **30**,146-155.

Pipa, A. V., Reuter, S., Foest, R. and Weltmann, K. D. (2012) Controlling the NO production of an atmospheric pressure plasma jet. *J. Phys. D: Appl. Phys.* **45**, 085201.

Polak, M., Winter, J., Schnabel, U., Ehlbeck J. and Weltmann, K. D. (2012) Innovative plasma generation in flexible biopsy channels for inner-tube decontamination and medical applications. *Plasma Process. Polym.* **9**, 67-76.

Reuter, S., Winter, J., Schmidt-Bleker, A., Schroeder, D., Lange, H., Knake, N., Schulz-von der Gathen, V. and Weltmann, K. D. (2012a) Atomic oxygen in a cold argon plasma jet: TALIF spectroscopy in ambient air with modelling and measurements of ambient species diffusion. *Plasma Sources Sci. Technol.* **21**, 024005.

Reuter, S., Winter, J., Iseni, S., Peters, S., Schmidt-Bleker, A., Dünnbier, M., Schäfer, J., Foest, R. and Weltmann, K. D. (2012b) Detection of ozone in a MHz argon plasma bullet jet. *Plasma Sources Sci. Technol.* **21**, 034015.

Robert, E., Vandamme, M., Brullé, L., Lerondel, S., LePape, A., Sarron, V., Riès, D., Darny, T., Dozias, S., Collet, G., Kiedaand, C. and Pouvesle, J. M. (2013) Perspectives of endoscopic plasma applications. *Clin. Plasma Med.* **1**, 8-16.

Salvioli, S., Ardizzoni, A., Franceschi, C and Cossarizza A. (1997) JC-1, but not DiOC6(3) or rhodamine 123, is a reliable fluorescent probe to assess Δψ changes in intact cells: Implications for studies on mitochondrial functionality during apoptosis. *FEBS Lett.* **411**, 77-82.

Schaper, L., Reuter, S., Waskoenig, J., Niemi, K., Schulz-von der Gathen, V. and Gans, T. (2009) The dynamics of radio-frequency driven atmospheric pressure plasma jets. *J. Phys: Conference Series* **162**, 012013.

Schmidt, A., von Woedtke, T., Weltmann, K. D. and Masur, K. (2013a) Identification of the molecular basis of non-thermal plasma-induced changes in human keratinocytes. *Plasma Med.* **3**, 15-25.

Schmidt, A., Wende, K., Bekeschus, S., Bundscherer, L., Barton, A., Ottmüller, K., Weltmann, K. D. and Masur, K. (2013b) Non-thermal plasma treatment is associated with changes in transcriptome of human epithelial skin cells. *Free Radic. Res.* **47**, 577-592.

Schmidt-Bleker, A., Winter, J., Iseni, S., Dünnbier, M., Weltmann, K.D. and Reuter, S. (2014) Reactive species output of a plasma jet with a shielding gas device-combination of FTIR absorption spectroscopy and gas phase modelling. *J. Phys. D: Appl. Phys.* **47**, 145201.

Shashurin, A., Stepp, M. A., Hawley, T. S., Pal-Ghosh, S., Brieda, L., Bronnikov, S., Jurjus, R. A. and Keidar, M. (2010) Influence of cold plasma atmospheric jet on surface integrin expression of living cells. *Plasma Process. Polym.* **7**, 294-300.

Shi, X. M., Zhang, G. J., Yuan, Y. K., Ma, Y., Xu, G. M. and Yang, Y. (2008) Effects of low-temperature atmospheric air plasmas on the activity and function of human lymphocytes. *Plasma Process. Polym.* **5**, 482-488.

Singh, N. P., McCoy, M. T., Tice, R. R. and Schneider, E. L. (1988) A simple technique for quantitation of low-levels of DNA damage in individual cells. *Exp. Cell Res.* **175**, 184-191.

Steinbeck, J., Chernets, N., Zhang, J., Kurpad, D. S., Fridman, G., Fridman, A. and Freeman, T. A. (2013) Skeletal cell differentiation is enhanced by atmospheric dielectric barrier discharge plasma treatment. *PLoS One* **8**, e82143.

Stoffels, E., Kieft, I. E. and Sladek, R. E. J. (2003) Superficial treatment of mammalian cells using plasma needle. *J. Phys. D: Appl. Phys.* **36**, 2908-2913.

Straβenburg S. (2014) Untersuchungen zum Einfluss von physikalischem biological auf in vitro kultivierte Zellen. *Doctoral Thesis*, Ernst-Moritz-Arndt-University of Greifswald

Straβenburg, S., Greim, U., Bussiahn, R., Haertel, B., Wende, K., von Woedtke, T. and Lindequist, U. (2013) Comparison of biological effects on human keratinocytes using different plasma treatment regimes. *Plasma Med.* **3**, 57-69.

Strausberg, J., Lehmann, N., Kröger, K., Maier, I., Schneider, H and Niebel, W. (2007) Changes in secondary care may explain increasing pressure ulcer rates in an University Clinic in Germany. *Wound Manag.* **5**, 194-198.

Sung, S. J., Huh, J. B., Yun, M. J., Chang, B. M., Jeong, C. M. and Jeon, Y. C. (2013). Sterilization effect of atmospheric pressure non-

thermal air plasma on dental instruments. *J. Adv. Prosthodont.* **5**, 2-8.

Taghizadeh, K., McFaline, J. L., Pang, B., Sullivan, M., Dong, M., Plummer, E. and Dedon, P. C. (2008) Quantification of DNA damage products resulting from deamination, oxidation and reaction with products of lipid peroxidation by liquid chromatography isotope dilution tandem mass spectrometry. *Nat. Protoc.* **3**, 1287-1298.

Vandamme, M., Robert, E., Lerondel, S., Sarron, V., Ries, D., Dozias, S., Sobilo, J., Gosset, D., Kieda, C., Legrain, B., Pouvesle, J.M. and Le Pape, A. (2012) ROS implication in a new antitumor strategy based on non-thermal plasma. *Int. J. Cancer* **130**, 2185-2194.

Volotskova, O., Hawley, T. S., Stepp, M. A. and Keidar, M. (2012a) Targeting the cancer cell cycle by cold atmospheric plasma. *Sci. Rep.* **2**, 636.

Volotskova, O., Stepp, M. A. and Keidar, M. (2012b) Integrin activation by a cold atmospheric plasma jet. *N. J. Phys.* **14**, 053019.

von Woedtke, T., Metelmann, H.R. and Weltmann, K.D. (2014) Clinical plasma medicine: state and perspectives of in vivo application of cold atmospheric plasma. *Contrib. Plasma Phys*. **54**, 104-117.

von Woedtke, T., Reuter, S., Masur, K. and Weltmann, K. D. (2013) Plasmas for medicine. *Phys. Rep.* **530**, 291-320.

Weltmann, K. D., Brandenburg, R., von Woedtke, T., Ehlbeck, J., Foest, R., Stieber, M. and Kindel, E. (2008) Antimicrobial treatment of heat sensitive products by miniaturized atmospheric pressure plasma jets (APPJs). *J. Phys. D: Appl. Phys.* **41**, 194008.

Wende, K., Straβenburg, S., Haertel, B., Harms, M., Holtz, S., Barton, A., Masur, K., von Woedtke, T. and Lindequist, U. (2014) Atmospheric pressure plasma jet treatment evokes transient oxidative stress in HaCaT keratinocytes and influences cell physiology. *Cell Biol. Int.* **38**, 412-425.

Werdin, F., Tennenhaus, M., Schaller, H. E. and Rennekampff, H. O. (2009) Evidence-based management strategies for treatment of chronic wounds. *ePlasty* **9**, e19.

Widgerow, A. D. (2013) Chronic wounds: is cellular 'reception' at fault? Examining integrins and intracellular signaling. *Int. Wound J.* **10**, 185-192.

Winter, J., Tresp, H., Hammer, M. U., Iseni, S., Kupsch, S., Schmidt-Bleker, A., Wende, K., Dünnbier, M., Masur, K., Weltmann, K. D. and Reuter, S. (2014) Tracking plasma generated H_2O_2 from gas into liquid phase and revealing its dominant impact on human skin cells. *J. Phys. D: Appl. Phys.* **47**, 285401.

Wu, Z., Chen, M., Li, P., Zhua, Q. and Wang, J. (2011) Dielectric barrier discharge non-thermal micro-plasma for the excitation and emission spectrometric detection of ammonia. *Analyst* **136**, 2552-2557.

Yu, Y., Tan, M., Chen, H., Wu, Z., Xu, L., Li, J., Cao, J., Yang, Y., Xiao, X., Lian, X., Lu, X. and Tu, Y. (2011) Non-thermal plasma suppresses bacterial colonization on skin wound and promotes wound healing in mice. *J. Huazhong Univ. Sci. Technol. Med. Sci.* **31**, 390-394.

Zhu, W. H., Iurlaro, M., MacIntyre, A., Fogel, E. and Nicosia, R. F. (2003) The mouse aorta model: influence of genetic background and aging on bFGF- and VEGF-induced angiogenic sprouting. *Angiogenesis* **6**, 193-199.

Zimmermann, J. L., Dumler, K., Shimizu, T., Morfill, G. E., Wolf, A., Boxhammer, V., Schlegel, J., Gansbacher, B. and Anton, M. (2011) Effects of cold atmospheric plasmas on adenoviruses in solution. *J. Phys. D Appl. Phys.* **44**, 505201.